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THROMBOSIS AND EMBOLISM*

AN ANALYSIS OF 1000 AUTOPSIES

BY SOL ROY ROSENTHAL, M.D., CHICAGO, ILL.

THE mechanism of the formation of thrombi has long been a matter of dispute. John Hunter (1784), who was first to have definite views on the subject, believed that infection altered the vessel wall and that a thrombus resulted as an exudation thereof. Cruveilhier (1829) thought that the clot was first to form and that the infection came secondarily. Andral (1842) modified the infectious theory by adding the factor of stasis. Virchow (1855) was the first to designate noninfectious processes in the causation of thrombi. His conception was that a sluggish circulation (marantic, compressional, dilatational and traumatic) in addition to various changes in the blood proper were predisposing factors. Baumgarten (1877) was able to produce clotting of blood in veins of animals by double ligations, only after he injured the vessel wall. (These were not typical thrombi.) Alexander Schmidt (1877) then demonstrated that clot formation was due to a fermentative influence of the constituents of the blood. Montagassa and Zahn (1875) believed that all thrombi were the result of coagulation and that the various colors and layers were due to secondary influences. (Later Aschoff, Beneke, De la Camp, Beck and Kronig, 1911.)

Bizzozero (1882) emphasized Hayem's work (1877) in relation to the rôle of platelets in clot formation and soon after Eberth and Schimmelbusch (1888) demonstrated that in a slow current, the platelets place themselves in

*From the Department of Pathology, Cook County Hospital, Dr. Richard H. Jaffé, Pathologist-in-chief.

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the outer edge of the current and become attached to the wall of the damaged vessel. The coagulation of the platelets leads to a platelet thrombus and fibrin, formed from the plasma, adds itself thereto. The rôle of erythrocytes and leucocytes they believed to be of minor importance. Zahn (1891), however, observed the deposit of white blood corpuscles on the injured vessel wall and distinguished this (white thrombus) from the thrombus formed by stagnation and coagulation (red thrombus). The latter is responsible for fatal lung emboli. Ribbert (1912) stated that a thrombus could not form without endothelial injury.

The present-day conception regarding the formation of thrombi stresses the physical, chemical or colloidal changes of the blood and sublimates endothelial injury and stasis (Dietrich (1920), Allen (1927), Höring (1928), Qure, 1929).

The recent rise in the incidence of thrombosis and embolism has been explained as the result of intensive heart therapy (Oberndorfer, Höring), intensive intravenous injections (von Linhard, Oehler, Fahr), betterment of nutrition and older age with heart and vessel changes (Kuhn).

A study has been made of the incidence of thrombosis and embolism in 1000 consecutive postmortem examinations during the year of 1929 and their relation to age, sex, race, clinics, surgical procedures, trauma, labor, infections, inflammations, nutrition and therapy.

Incidence.—Of the 1000 postmortem examinations there were 134 cases of thrombosis, 76 cases of embolism and 2 cases of fatal lung embolism. In

TABLE I

THE INCIDENCE OF THROMBOSIS, EMBOLISM AND FATAL LUNG EMBOLISM IN THE VARIOUS CLINICS IN EUROPE AND THE UNITED STATES

CLINIC	YEAR	AUTOPSY NO.	THROMBOSIS		EMBOLI		FATAL LUNG EMBOLI	
			NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
General								
Rupp (Chemitz)	1921	12,971					657	5.0
Nageli Bonn (Inst. Monckeberg)	1925	4,916					43	0.87
Oberndorfer (Munich)	1925-27	2,500	300	12.0			125	5.0
Höring (Univ. Rostock)	1927	395					27	6.8
Kuhn, J. K. (Univ. Freiburg)	1927	466	114	24.5	58	12.5	23	4.94
Morawitz (Singer) Leipzig	1926-28	3,400	1,020	30			221	6.5
Rostock	1927	3,248					131	4.0
McCartney (Minn.)	1927	9,275					73	0.79
Lubarsch (Possen)		2,000	640	32				
Lubarsch (Kiel)		3,634	863	21.48				
Stohr-Kazda (Vienna)		20,654					134	0.56
Stohr-Kazda (Vienna)		714					13	1.9
Stohr-Kazda (Vienna)		2,664					13	0.49
Krauspe (Leipzig)		5,500	165	3-4			2	0.04
Surgical								
Henderson (Rochester)	1917-27							6.0
Sulger-Bozsin (Heidelborg Sur. Cl.)	1926	177					24	13.5
Bauer (Wurzborger Sur. Cl.)	1928			1.15				0.41
Payr (Leipzig Sur. Cl.)		30,195					1,117	3.7
Average per cent				*17.7*		*12.5*		*0.96*
Cook County Hospital	1929	1,000	134	*13.4*	76	*7.6*	2	*0.2*

comparing these results with those of other authors a wide variation is noted, because different types of clinical material are reported and different pathologists evince varying interest in these conditions. However, when an average is taken from all the reports, the similarity is striking. Thus the average for thrombosis is 17.7 per cent, embolism 12.5 per cent, and fatal lung embolism 0.9 per cent as compared to 13.4 per cent, 7.6 per cent and 0.2 per cent as represented by the material reported. (Table I.)

Age.—The largest number of thrombi (37) occurred between forty-one and fifty-one years of age. (This is 27 per cent of the total number of thrombi [specific percentage].) However, the largest number of postmortem examinations (220) were performed in this group, so that calculating on the basis of 100 cases, the percentage drops to 16.8 per cent (proportionate percentage).* By this method of calculation, the greatest percentage of thrombosis (28.8 per cent) occurred at ages of seventy-one and over, whereas for embolism the greatest percentage (16.2 per cent) fell between fifty-one and sixty years. The two cases of fatal lung embolism were in patients between sixty-one and seventy years. (Table II.)

TABLE II

THE RELATION OF AGE TO THROMBOSIS, EMBOLISM, AND FATAL LUNG EMBOLISM

AVERAGE FOR 1000 P.M.			THROMBI (134)			EMBOLI (76)			FATAL EMBOLI (2)		
AGE	NO.	PER CENT	NO.	SPEC. PER CENT	PROP. PER CENT	NO.	SPEC. PER CENT	PROP. PER CENT	NO.	SPEC. PER CENT	PROP. PER CENT
0-5	160	16.0	6	4.4	3.7	1	1.3	0.62	0	0	0
5-10	45	4.5	1	0.7	2.2	0	0	0	0	0	0
11-20	65	6.5	3	2.2	4.6	2	2.6	3.0	0	0	0
21-30	105	10.5	9	6.7	8.5	3	3.9	2.8	0	0	0
31-40	135	13.5	21	15.6	15.5	12	15.7	8.9	0	0	0
41-50	220	22.0	*37*	*27.7*	16.8	*26*	*34.2*	11.8	0	0	0
51-60	135	13.5	33	24.6	24.5	22	28.9	*16.2*	0	0	0
61-70	90	9.0	11	8.2	12.2	5	6.5	5.5	*2*	*100*	*2.2*
71-over	45	4.5	13	9.9	*28.8*	5	6.5	11.1	0	0	0

Taken together, 70.4 per cent of all thrombi and 76.1 per cent of all emboli occur after forty-one years of age. These results are similar to those found by other authors. (Bull, Kuhn, Klinge, Morowitz and Singer, Oberndorfer, Höring.)

Sex.—The male represents by far the largest number of thrombosis (107 out of 134 or 79.8 per cent) as compared to the female (27 or 20.1 per cent). Considering the number of male and female postmortems, however, the relation is 700 to 300 in favor of the male. The proportionate percentage, or the percentage in 100 autopsies then reads, for the male, 15.2 per cent and for the female, 9 per cent. Nonfatal embolism shows a similar relation. The two cases of fatal lung embolism were in females. (Table III.)

Kuhn has shown a definite increase of thrombi in the female over the male (1.4 to 1). Fahr and Hueck believe that sex does not play a noteworthy

*Where applicable the studies in this report have been considered in both specific and proportionate percentages.

TABLE III

THE RELATION OF SEX TO THROMBOSIS, EMBOLISM, AND FATAL LUNG EMBOLISM

	THROMBOSIS (134)			EMBOLISM (76)			FATAL EMBOLISM (2)		
	NO.	SPEC. PER CENT	PROP. PER CENT	NO.	SPEC. PER CENT	PROP. PER CENT	NO.	SPEC. PER CENT	PROP. PER CENT
Male (700 cases)	107	79.8	*15.2*	61	80.2	*8.7*	0	0	0
Female (300 cases)	27	20.1	9.0	15	19.7	5.0	2	100	*0.66*

rôle. For fatal lung embolism, all authors agree that the female sex has the predominance. (Höring, Oberndorfer, Singer, Kuhn.)

The reasons given for the female predominance are birth trauma, abortions, diseases of the sex organs and puerperal infections. It is at once obvious that clinics in which there is a great percentage of such cases will report conflicting results with those of other types of clinics. Thus of the 1000 postmortems, there were only 30 cases or 3 per cent that came from the gynecologic and obstetric services.

In summary then, the male sex shows the larger percentage (both specific and proportionate) of thrombosis and nonfatal embolism while the female sex included the two cases of fatal lung embolism.

Race.—When considering proportionate percentage, the incidence of thrombosis and embolism are about equal in white and colored. (Table IV.)

TABLE IV

THE RELATION OF RACE TO THROMBOSIS, EMBOLISM, AND FATAL LUNG EMBOLISM

	THROMBOSIS			EMBOLISM			FATAL LUNG EMBOLISM		
	NO.	SPEC. PER CENT	PROP. PER CENT	NO.	SPEC. PER CENT	PROP. PER CENT	NO.	SPEC. PER CENT	PROP. PER CENT
White (610 cases)	83	61.9	13.6	48	63.1	7.8	1	50.0	0.16
Colored (390 cases)	51	38.0	13.3	28	36.7	7.2	1	50.0	0.25

Clinics.—The greatest number of thrombi (110) and emboli (60) were derived from the medical clinics. This is a specific percentage of 82 and 76.9, respectively. When cognizance is taken of the fact that 610 of the 1000 postmortems came from medical wards, the percentage drops to 18. By this procedure the surgical eye, ear, nose and throat cases represent the highest proportionate percentage of thrombosis (30 per cent) and the obstetric cases the highest proportionate percentage of embolism (20 per cent). (Table V.)

This is not conclusive as will be shown later; many of the patients dying in surgical wards were not operated upon and had associated heart conditions.

Nonoperative and Operative Procedures Including Trauma and Labor.—By classifying the cases according to those without operation and those with operation, trauma or labor, it was found that the largest number of thrombi

TABLE V

THE RELATION OF THROMBOSIS AND EMBOLISM TO THE VARIOUS DEPARTMENTS OF MEDICINE

	THROMBOSIS			EMBOLISM		
	NO.	SPEC. PER CENT	PROP. PER CENT	NO.	SPEC. PER CENT	PROP. PER CENT
Medical (610 cases)	110	*82.0*	18.0	60	*76.9*	9.8
Surgical (150 cases)	8	5.9	5.3	7	9.2	4.6
Pediatrics (190 cases)	8	5.9	4.0	1	1.3	0.5
Gynecology (10 cases)	2	1.48	20.0	0	0	0
Genitourinary (10 cases)	1	0.74	10.0	1	1.3	10.0
E. N. T. (10 cases)	3	2.2	*30.0*	1	1.3	10.0
Obstetrics (15 cases)	2	1.48	13.3	3	3.9	*20.0*

(122) and emboli (62) were found in the nonoperative group. The highest proportionate percentage for thrombi was also in this group (13.6 per cent), but for emboli, it was in the operative group (11.4 per cent). This group included 7 postoperative cases, 4 postpartum cases and 1 fracture case.* Each group had one fatal lung embolism. (Table VI.)

TABLE VI

THE RELATION OF THROMBOSIS AND EMBOLISM TO SURGICAL PROCEDURES, TRAUMA, AND LABOR

	THROMBOSIS			EMBOLISM			FATAL LUNG EMBOLISM		
	NO.	SPEC. PER CENT	PROP. PER CENT	NO.	SPEC. PER CENT	PROP. PER CENT	NO.	SPEC. PER CENT	PROP. PER CENT
Cases without operation. (895 cases)	122	*91.0*	*13.6*	62	*81.5*	6.9	1	50.0	0.12
Operative cases. Trauma. Labor. (105 cases)	12	8.9	11.4	12	15.8	*11.4*	1	50.0	*0.9*

In analyzing the 12 cases of thrombi and emboli in the operative group, it was found that 8 of them had advanced changes in the heart and arteries which were obviously preoperative.

The case of fatal lung embolus in the operative group followed 18 days after a fracture of the left femur. A thrombus was found at the site of the fracture. In addition, there was an eccentric hypertrophy of the heart (405 gm.) with coronary sclerosis and phlebosclerosis.

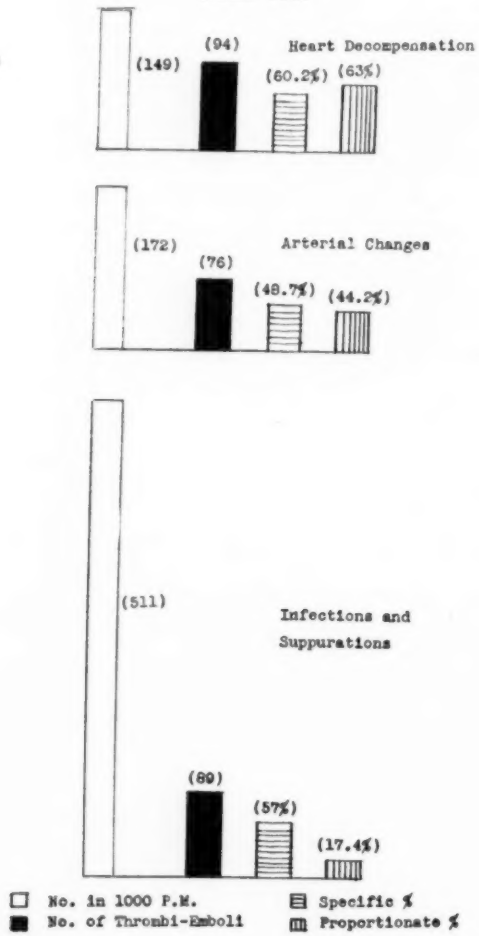
McCartney has emphasized the frequency of trauma as an etiologic factor in the formation of fatal lung emboli. He found that out of 73 cases of fatal lung embolism, 23 followed trauma. Other authors (Sulger and Bozsin, Loewe, Prochnow, Morawitz, Henderson) report a high incidence of fatal lung embolism following operative procedures. In the two cases reported, neither followed such procedures.

*This was the only traumatic case in the entire series as these cases are turned over to the coroner's office.

In summarizing, the highest proportionate percentage of thrombi occurred in the nonoperative group, of emboli, in the operative group. Of the latter group, the majority were associated with heart and vascular changes.

Cardiac, Vascular Disease, Infection and Inflammation.—Under heart, only those cardiac conditions were considered in which there were definite signs of cardiac failure. Under arterial changes, arteriosclerosis and syphilis were

TABLE VII.



considered, but only the more prominent was recorded if the two conditions coexisted. Under infections and inflammations only those were considered that were of longer standing than the thrombosis or embolism; thus a terminal bronchopneumonia was not included.

Of the 149 cases of decompensation of the heart in the entire series of 1000 cases there were 94 with thrombosis or 63 per cent; of the 172 cases with arterial changes, 76 or 44 per cent had thrombi and of the 511 infectious cases 57 or 17.4 per cent had thrombi. Apparently cardiac decompensation and arterial changes play the more important rôle in the formation of thrombi

and emboli. These conclusions are not evident unless the calculations are based per 100 cases, for of the 156 thrombi-emboli (*considered as one*) there were 89 or 57 per cent with infections. When one considers, however, that there were 511 cases with infections or suppurations in 1000 postmortems, the proportional percentage drops to 17.4 per cent. (Table VII.)

The two cases of fatal lung emboli were associated with cardiac decompensation. Of these, one had in addition arterial changes while the other had a purulent cystitis and pyelitis (no temperature).

In summary, cardiac and vascular changes are more frequent in their association with thrombosis and embolism than infectious and suppurative conditions.

Heart and Arteries.—By grouping the cardiac conditions and arterial changes together, they constitute 119 cases associated with thrombi-emboli formation or 76.2 per cent of the total number of thrombi-emboli.

Hypertrophy, atrophy and scarring of the myocardium make up 63 per cent, 8.4 per cent, and 18.4 per cent, respectively, of the 119 cases. Collectively, this group makes up 89.8 per cent.

Arteriosclerotic and syphilitic changes make up 37.9 per cent and 24.3 per cent, respectively. Collectively, this is 62.2 per cent. (Table VIII.)

TABLE VIII

THE RELATION OF THROMBOSIS AND EMBOLISM TO CHANGES IN THE HEART AND ARTERIES

	NO.	PER CENT OF 119
Heart:		
Hypertrophy-dilatation-insufficiency with decompensation	75	63.0
Atrophy with decompensation	10	8.4
Disease of coronary vessels with scarring of myocardium with decompensation	10	8.4
Endocarditis	22	18.4
Pericarditis	3	2.5
Vessels:		
Arteriosclerosis	47	37.9
Syphilis	29	24.3
Cerebral vascular disease	11	9.2

From these results, cardiac decompensation predisposes more frequently to thrombi-emboli formation than does arterial change.

Infections and Inflammations.—The infectious processes such as pneumonia, typhoid, diphtheria, and tuberculosis have been separated from the suppurative processes such as phlegmon, otitis media, appendicitis, peritonitis, pyemia, septicemia, and puerperal sepsis. When considered as two groups, the infectious processes have 49 cases as compared to 40 cases of the suppurative group. Of the latter, the local conditions causing thrombi, such as otitis media with secondary sinus thrombosis, or endometritis and cystitis with thrombosis of the hypogastric vein, were 17 in number as compared to 19 cases in which the suppuration was remote from the thrombus. Thus, in 68 out of a total of 89 cases, thrombosis was found as a result of a generalized infection or a remote suppuration. (Table IX.)

TABLE IX

THE RELATION OF THROMBI-EMBOLI TO INFECTIONS AND INFLAMMATIONS

	INFECTIONS		INFLAMMATIONS					
			PROXIMAL TO THROMBUS		REMOTE FROM THROMBUS		PUERPERAL SEPSIS	
	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Thrombi-Emboli (156)	49	31.4	17	10.9	19	12.1	4	2.5
Fatal Lung Emboli (2)	0	0	1	50	0	0	0	0

Of the 89 cases there were 60 which were associated with heart and vessel changes, leaving only 27 cases in which infection alone was responsible for thrombus formation, or a proportional percentage of 5.

In puerperal sepsis the incidence of thrombosis was very high. Thus out of 5 cases there were 4 with thrombosis. Duffek, however, has demonstrated that in puerperal infection, thrombosis occurs primarily and infection is secondary, for normally the placenta is studded with thrombi.

In summarizing, of the 1000 postmortems, infections and inflammatory diseases played a minor rôle in the formation of thrombi and emboli. The generalized infections and the remote suppurations had a greater incidence of thrombosis than the local processes.

Nutrition.—Because the general nutrition has been improved since the war, and because the incidence of thrombosis and embolism has increased, various authors (Deider, Kuhn) have attempted to correlate the two. Others have noted that the majority of fatal lung emboli occur in obese individuals. (Henderson, Snell.) Snell has explained the coincidence on an increased liberation of a thromboplastic lipoid substance. He found that true fat emboli were rare (2 out of 56 cases).

In this study, the greatest number of thrombi (79), emboli (54) and fatal lung emboli (2) occurred in the well nourished group. (Table X.)

TABLE X

THE RELATION OF THROMBOSIS AND EMBOLISM TO NUTRITION

	THROMBOSIS (134)	EMBOLISM (76)	FATAL LUNG EMBOLISM (2)
Well nourished			
Good	43	33	0
Very good	29	16	1
Obese	7	5	1
Total	79 (59%)	54 (71%)	2 (100%)
Under nourished			
Thin	34	16	0
Emaciated	16	4	0
Cachectic	5	2	0
Total	55 (41%)	22 (29%)	0 (0%)

Here again the factor of heart and vessels is noteworthy, as 84 per cent of the well nourished, as compared to 64 per cent of the undernourished were associated with heart and vascular changes.

In summarizing, the well nourished group have the higher percentage of thrombi, emboli and fatal lung emboli, but also the highest percentage of heart and vascular changes.

Therapy.—The newer methods of intensive heart therapy are prevalent at the Cook County Hospital as elsewhere. Of this series of cases, 25 per cent received digitalis or one of the various diuretics. Although this is but a small percentage, the cases receiving the most intensive treatment showed the most marked thrombus formation, especially in the heart. The chambers of the heart were usually dilated and filled with extensive thrombi.

The relation to intravenous and subcutaneous injection therapy has been omitted because so few patients die as a result of the therapy.

DISCUSSION

From this study, the highest incidence of thrombosis and embolism occurred in individuals over forty-one years, in whom cardiac conditions were most prominent.

Can one infer then, that stasis of the blood, resulting from a failing myocardium, is responsible for thrombus formation?

Roger and Miller, repeating Baumgarten's work of double ligation, searring of the endothelium, interposing muscle bellies, in the veins of cats, were unable to produce typical thrombi.

From these experimental findings, stasis alone, or in association with endothelial injury, will not result in the formation of thrombi. It follows then that alterations in the blood itself are prerequisite. The nature of these changes and the manner in which they are brought about is still an open question.

In the past decade, the expectant length of life has increased from fifty-five to sixty-one years (I. Fisher). With older age, the destructive processes of the body are exaggerated. (The descending stage of Aschoff.) These changes affect the blood as well as other parts of the body. The exact nature of these changes, whether they be chemical, physical or colloidal, is not known. The superimposing of myocardial failure in such an individual, with the resulting stasis and acidosis (Friedmann) will in the majority of instances, as has been shown by this study, lead to thrombus formation. If arterial changes are also present, the additional factors of endothelial injury further alter the blood (increase of calcium and cholesterol in arteriosclerosis, Gechtman and Slauskayu) and augment the susceptibility to thrombus formation.

Thus is would seem that the factors of stasis and endothelial injury, although important, are ineffectual unless changes in the blood are first manifest.

Other authors have shown that changes in the blood leading to thrombus formation result from infection (Dietrich, Höring, Miller and Rogers); from operative procedures (Qure, von Seeman, Sulger and Bozsin, Allen).

Although infectious processes and suppurations may lead to thrombosis, the incidence in this study was much less than thrombosis caused by non-

inflammatory conditions. It was of interest to note, also, that 76 per cent of the thrombi associated with infections or suppurations were remote from the site of the infection, so that the factor of a generalized blood change was again prominent.

Changes in the blood following operative procedures as a factor in thrombus formation have been stressed by Qure, who found an increase in calcium; by Sulger and Bozsin, who found that the clotting and bleeding time was shortened in two-thirds of the cases; by Allen and Höring, who speak of chemical colloidal changes; by von Seeman who found an increase in the globulin and fibrinogen content of the blood.

Of the 1000 postmortem examinations, there were 89 postoperative cases. Out of this group 7 were associated with thrombi-emboli (or 7.8 per cent). The average age of this group was forty-four years. In six of the seven cases, changes of the heart and vessels were in evidence. There again one was dealing with an older individual in which heart and vessel changes were prominent.

Trauma as a factor in the production of fatal lung embolism has been emphasized by McCartney and De la Camp. The latter found an excessive amount of kinase thrown into the blood following trauma. He believes this factor of utmost importance in the formation of thrombi. In the case of fatal lung embolus following trauma, the age of the patient and the advanced heart and vessel changes cannot be overlooked as a possible cause, the trauma acting as the exciting factor in a susceptible patient.

CONCLUSIONS

1. The incidence of thrombi and emboli in 1000 postmortem examinations is discussed and its relation to age, sex, color, clinics, heart disease, infectious and suppurative diseases, operative procedures, puerperium, and therapy is elaborated upon.

2. The mechanism of the formation of thrombi and emboli as brought out in the literature and this statistical report, is dependent upon a colloidal, chemical or physical alteration of the blood, in association with, in order of importance, heart and vessel changes, and infectious processes.

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ENDOCRINE CORRELATIONS*

The Embryohormonic Relations of the Suprarenal Cortex to Mesothelial Tissues

By R. C. Moehlig, M.D., Detroit, Mich.

THE greatest handicap that workers with endocrine glands have found has been that of interglandular relationships. That embryology supplies the solution to many of the correlations has been my contention for many years. Certain endocrine glands have a selective action on tissues depending upon their embryologic origin. This selective action of glandular secretions has been termed embryohormonic in order to emphasize the effect the secretions have on tissues according to their embryologic origin. Teaching the relationships according to the embryohormonic idea makes the understanding of endocrine function and symptomatology much easier and paves the way for solving many obscure problems.

It is to be emphasized that the endocrine gland in question does not necessarily have a selective action on the tissue from which it originates. For instance, the pituitary gland, of ectodermal origin, does *not* have a selective action on ectodermal tissues.

The fundamentals laid down in my original work[1] appear correct and further work has strengthened them. Because the embryohormonic relations of the suprarenal cortex are comparatively simple, this will be used as an illustration.

The suprarenal cortex is derived from the mesoderm, in contrast to the medulla which is an ectodermal tissue. The cortex influences the tissues derived from a special part of the mesoderm, called the mesothelium. From this is derived the following tissues:

(1) Ovary, Testicles, Sex Organs } Gonads
(2) Voluntary muscle system (Including the specialized cardiac muscle)

The pleura, pericardium and peritoneum are also derived from the mesothelium but we need not discuss them here. Since we assume a selective ac-

*From the Department of Internal Medicine, Harper Hospital, Detroit, Michigan.
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tion of the suprarenal cortex secretion on these tissues, changes in its function will be manifested in these tissues. This is best illustrated by primary pathology in the suprarenal cortex. A clue to its physiologic function is furnished by gross degrees of hypo- and hyperfunction. Hypofunction is best illustrated by Addison's disease.

Some of the data which show the relationship of the suprarenal cortex to the mesothelial gonads and sex organs will be given first. This relationship is very definite.

In Addison's disease Kyrle[2] found deficient spermatogenesis and degenerative changes in the interstitial glands. Impotency in the male and amenorrhea in the female is present and the testicles and ovaries are atrophic.

Leupold[3] found a close relationship existing between the suprarenal and testicles. He found that when the suprarenals contain much fat the quantity of fat in the testes is also high. The testes contain less cholesterol than do the suprarenals and when cholesterol diminishes in the testes and the suprarenals it is generally first decreased in the former. He believes this proves that the suprarenals have a "higher" function than the testes.

Congenital aplasia of the suprarenal cortex such as accompanies the pituitary aplasia seen in acrania, has a concomitant aplasia of the gonads and sex organs. Marked malformation of the genitals is present. As early as 1806, Meckel[4] called attention to the relationship in size between the well-developed suprarenals and genital organs of the guinea pig. More recently Leupold[5] shows that the weight relationship of the suprarenals and testicles parallel one another. Mulon,[6] Kolmer,[7] and others have shown the histologic resemblance between the lipoid containing cells of the suprarenal cortex and the corpus luteum.

During rut and pregnancy the suprarenals enlarge, likewise castration produces enlargement of the suprarenal, particularly the cortex.

Leinati[8] found in castrated rabbits and guinea pigs that the cortex became hypertrophic and likewise the accessory suprarenals were macroscopically enlarged.

Kichikawa's[9] experiments point to a stimulating influence of the suprarenals on the sex characteristics.

Stewart and Rogoff[10] found that pregnancy conferred a certain degree of protection to suprarenalectomized dogs. This would confirm Mulon's opinion that the corpus luteum of pregnancy is a temporary suprarenal cortex. Leupold[5] is of the opinion that the suprarenals influence the function of the sex organs and libido through the intermediary of the cholesterol metabolism.

Hoskins and Hoskins[11] found hypertrophy of the testes and ovaries in white rats which had been fed desiccated suprarenal substance. Vigor of reproduction is reduced by partial suprarenal extirpation. (Vincent and Thompson.[12])

Novak[13] found that suprarenal extirpation in rats, mice, and female dogs resulted in disappearance of the estrous cycle and degeneration of the follicles.

In hyperfunction of the suprarenal cortex as exemplified in hypernephromata, sexual precocity and overdevelopment is present. Females of one and one-half to two years menstruate and develop the condition of pubertas pre-

cox. The literature is full of cases of this type and likewise the association of suprarenal cortex hyperplasia associated with intersexuality. There is no need for us to dwell further on this relationship. Bauer[14] and many others have covered these aspects of suprarenal cortex function. We are safe in saying that the suprarenal cortex hormone stands in close physiologic relation to the sex glands and their function.

It remains to show that the suprarenal cortex has a selective action on the mesothelial skeletal muscle system including the cardiac muscle.

Today it is generally accepted that the cortex and not the medulla of the suprarenals is essential to life. The work of Stewart and Rogoff[10] has done much to clear up this point.

Hypofunction of the suprarenals, such as seen in Addison's disease, results in intense muscular asthenia. This is of course characteristic of the disease. Biedl's extirpation experiments[15] on the fish species, skates and rays give very strong support to the opinion that the suprarenal cortex influences the skeletal musculature. In this species of fish the medulla and cortex are anatomically separate. Excision of the interrenal (cortex) tissue produced striking symptoms of muscular asthenia. Symptoms began seven to eight days after extirpation, consisting of lessened movements and lifelessness. Fourteen to eighteen days after extirpation, the muscular asthenia became so pronounced that the fish were unable to swim.

Elliott and Tuckett[16] from their observations, concluded that the cortical portion has a definite relation to the skeletal musculature. They noted that the suprarenals increase with the development of the skeletal muscles, the increase affecting mainly the cortex. They also found that the cortex parallels the weight, whereas the medulla remains stationary. Severe exhaustion is always associated with hemorrhagic dilatation of the blood spaces in the cortex. Bardier and Bonne[17] in studying the modifications produced in the structure of the suprarenals by tetanization of the muscles found that these affected the cortex of the gland, not the medulla.

Vincent and Thompson[12] say that several workers have adduced evidence that the cortex has some function in connection with muscular activity and their experiments point to the conclusion that some substance which they propose to call "pneumin" essential for the normal movements of respiration, is secreted by the suprarenal cortex, passes through neighboring lymphatic nodes and into veins by way of the thoracic duct and the right lymphatic duct.

Kühl[18] found that in suprarenalectomized guinea pigs, intravenous injections of suprarenal cortex extract restored the integrity of the asthenic skeletal muscles for a time and the hyperpnea disappeared for a longer time. Likewise in suprarenalectomized frogs and selaceans it has been found that suprarenal cortex injections improve the muscle integrity. Therefore, the suprarenal cortex would appear to secrete a hormone essential to muscle integrity (Trendelenburg[19]).

de Mira and Fontes[20] have reviewed the subject in regard to the muscular activity and the suprarenal cortex and added their own observations. These authors conclude that the substance whose action shows itself by muscular

weakness in suprarenalectomized animals is not epinephrine which they affirm to be positively harmful to muscular contraction. They are further of the opinion that the active principle exerts its influence locally on the muscle.

Bauer[14] says the regularity with which the paralysis of the hind extremities follows suprarenal cortex extirpation in fish and frogs up to the highest vertebrates, speaks for a fundamental vital function of the suprarenal cortex for the integrity of muscle. Animals, and particularly rats which have accessory suprarenals and survive bilateral suprarenalectomy without symptoms, show their suprarenal insufficiency after muscular exertion. They fatigue easily and may die suddenly. Epinephrine does not overcome this muscular asthenia.

Erni[21] found that juice expressed from the muscles of suprarenalectomized rats when injected into other suprarenalectomized rats produces almost fatal asthenia but has no influence on normal rats. He also found poisonous products in the muscles of suprarenalectomized dogs which were absent in normal animals.

Elliott[22] says that the striped muscle is continuously under a tonic influence possibly in part derived from nerve impulses, but such a condition as myasthenia gravis proves that the tone is not altogether supplied from the central nervous system. He ascribes this tonic influence to the suprarenal cortex secretion.

Castaldi[23] found that feeding suprarenal cortex of the ox to the very young guinea pig favors the formation of muscle.

Concerning the cardiac muscle: here we have a reasonable explanation of the hypotension in Addison's disease and in other conditions in which the suprarenal cortex is atrophic. The cardiac muscle shares in this asthenia thus accounting for the low blood pressure. Rogoff[24] says: "The low blood pressure in Addison's disease which has hitherto been interpreted as an indication of interference with the epinephrin secretion from the adrenals we believe is probably a result of deficient cortical function, perhaps resulting from an influence of the gradually developing intoxication upon the circulatory organs. In view of the better experimental and clinical studies we must consider Addison's disease as a manifestation of disease of the interrenal tissue rather than the chromaffin tissue of the adrenal glands."

It is of interest that in aplasia of the suprarenal cortex such as accompanies acrania, there is a concomitant hypoplasia of the cardiac muscle, as well as poorly-developed skeletal muscles. In hyperplasia of the suprarenal cortex such as we see in tumors, a marked development of the skeletal muscle takes place resulting in great muscular strength. Children who have these tumors are known as infant Hercules.

Pertinent to the present discussion which bears out the selective action of the suprarenal cortex on mesothelial tissues, is the relationship between the pituitary gland and the suprarenal cortex. In several previous articles[25] it was shown that the state of the pituitary gland is reflected or mirrored in the suprarenal cortex. Aplasia or atrophy of the pituitary results in a concomitant aplasia or atrophy of the suprarenal cortex, whereas hyperplasia of the pituitary results in hyperplasia of the suprarenal cortex.

Osius and I in a series of experiments on rabbits found that injection of posterior lobe extract resulted in enlargement of the suprarenals. The average weight of the suprarenals in the controls was 278 mg., the weight of the injected group was 415 mg.; that of the rabbits on a high fat diet was 435 mg.; and the suprarenals of the high fat diet plus posterior lobe injections was 639 mg. These experiments were conducted over a course of one hundred days. We will report elsewhere our findings in detail.

Since this is true then disturbances of the pituitary would show themselves in the mesothelial tissues. There is no need to dwell upon the obvious proof of this, examples of which are furnished by experimental hypophysectomy, pituitary dwarfism, dystrophia adiposogenitalis, with the accompanying hypoplasia of the suprarenal cortex and mesothelial tissues. Hyperplastic states of the pituitary result in changes in the suprarenal cortex and mesothelial tissues. A beautiful example of this is the case of experimental acromegaly produced by Putnam, Benedict, and Teel.[26] They used anterior lobe injections which resulted in hyperplasia and adenoma formation of the suprarenal cortex and likewise hypertrophic changes in the ovaries and skeletal muscle.

That Nature has provided us with a wonderful correlated mechanism is seen in many ways. For instance the hyperplastic pituitary of acromegaly and gigantism results among other things in a huge bony skeleton, and Nature provides a hyperplastic suprarenal cortex with a commensurate enlargement of the mesothelial skeletal muscle system to take care of this.

It is rather interesting that castration experiments followed by enlargement of the suprarenal cortex, could readily be understood as an attempt on the part of Nature to overcome this by pituitary hyperplasia with resulting suprarenal cortex hyperplasia.

My purpose has been to merely outline the principles of the embryohormonic relations of the suprarenal cortex to mesothelial tissues. The whole relationship fits in like a mosaic pattern and furnishes an easy understanding of many endocrine problems.

SUMMARY

Embryology supplies an easy understanding of interglandular relationships. This particular paper deals with the embryohormonic relations of the suprarenal cortex to mesothelial tissues.

By embryohormonic control is meant the selective action of endocrine secretions on tissues depending upon their embryologic origin.

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SYSTEMIC BLASTOMYCOSIS*

By Rigney D'Aunoy, M.D., and J. L. Beven, M.D., New Orleans

SYSTEMIC blastomycosis is no longer considered a rarity in most parts of this country, due undoubtedly, to the stimulus given its study by the many excellent case reports and reviews of the condition that have appeared in medical literature during the last two decades.

Usually, the occurrence of visceral blastomycotic involvement is secondary to cutaneous lesions caused by the parasites; but such cases as Ferguson's, Fairfield and Kispert's, Cleland's, Medlar's Case 2 as well as one herein recorded, show that systemic involvements need not necessarily be secondary to cutaneous manifestations, or at least can occur as sequelae to superficial lesions not sufficiently grave to cause notice thereof to be taken.

From 1906 to 1929 the diagnosis of "blastomycosis" appears 46 times in the records of the Charity Hospital of New Orleans. Analysis of the histories bearing this diagnosis shows that in only 26 of the cases, was the causal organism demonstrated. Of these authenticated cases, 16 showed only cutaneous lesions; nine, systemic involvements as well as cutaneous lesions, and one, systemic lesions with no discernible cutaneous involvement. Table I tabulates the essential features of these cases.

The histopathology in a number of the cases showing systemic involvement was intensely studied. As Case 26 showed clinical features of interest, and as its histopathology can be regarded as typical of the visceral lesions found in all cases of the series, it appears desirable to here record its salient features in detail.

L. R., colored male, aged fifty-five, laborer, was admitted, complaining of cough and weakness. His present trouble had begun two and a half months previously, with cough and much yellowish, thick, tenacious sputum. About two weeks before admission he began having night sweats. Since the onset of his cough he had noticed increasing muscular weakness and for the preceding five weeks had been unable to do any work. His past and familial histories were of no import.

Physical Examination.—Revealed a well developed, poorly nourished, male with few carious teeth. There was no glandular enlargement. His chest showed marked depression of the supraclavicular spaces with greatly diminished expansion of both lungs, especially the left. Percussion elicited dullness and flatness over the whole of the left side and the upper part of the right side. Crepitant râles were heard over both apices and over the whole extent of the left lung. Nothing further was revealed by physical examination. A provisional diagnosis of pulmonary tuberculosis was made on the strength of these findings.

Laboratory Examinations.—The Wassermann reaction of the blood was negative. The urine showed many hyaline and granular casts. There was a moderate secondary anemia with slight lymphocytosis. Repeated examinations of the sputum failed to reveal acid-fast organisms but there were present many budding and nonbudding yeast-like bodies. These

*From the Department of Pathology, Charity Hospital, New Orleans, La.
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TABLE I

CASE	AGE	SEX	COLOR	LOCATION	HISTOBIOLOGIC DIAGNOSIS	RESULTS
1	21	M	C	Right eye; right leg	Smears showed blastomycetes	Improved
2	62	M	W	Lesions of face and scalp; systemic involvement	Blastomycetes found in sections from face and scalp	Died. Autopsy showed blastomycosis of skin, bones, brain, lungs, spleen, pericardium, and heart
3	32	M	W	Lesions of trunk, extremities, and lungs	Blastomycetes found in tissue from chest sinus and face; also in pus from leg and sputum	Died; no autopsy
4	42	M	W	Right hand	Blastomycetes found in sections	Improved
5	58	M	C	Face	Smears showed blastomycetes	Improved
6	24	M	C	Lower left eyelid; right thigh; right arm	Blastomycetes found in scrapings	Improved
7	60	M	W	Left arm and hand	Blastomycetes found in scrapings	Improved
8	43	M	W	Right axilla	Blastomycetes found in scrapings	Died. Autopsy showed cutaneous blastomycosis and pulmonary blastomycosis
9	32	M	C	Neck	Blastomycetes found in smears from lesions	Improved
10	41	M	W	Right hand	Blastomycetes found in smears	Improved
11	?	M	C	Jaw	Smears showed blastomycetes	Died; no autopsy
12	40	M	W	Lungs; forehead	Scrapings from face showed blastomycetes	Died. Autopsy showed blastomycosis of the lungs and face
13	23	M	C	Lungs; skin	Blastomycetes found in sputum	Deserted; unimproved
14	29	M	C	Cheek; neck; chest	Blastomycetes found in sections	Improved
15	37	M	C	Chest; neck	Blastomycetes found in scrapings	Improved
16	43	M	W	Left arm	Blastomycetes found in sections	Improved
17	71	M	W	Right forearm	Blastomycetes found in sections	Improved
18	55	F	C	Face	Scrapings showed blastomycetes	Died; no autopsy
19	32	M	C	Lip	Blastomycetes found in sections	Improved
20	51	M	W	Cheek, nose, and lungs	Tissue from cheek shows blastomycetes; sputum shows blastomycetes in smears and cultures	Improved
21	24	M	C	Nose, face, and lungs	Blastomycetes in smears and cultures from nose, cheek, and pleural fluid	Died; no autopsy
22	?	F	C	Right leg; both hands; face	Blastomycetes found in sections	Improved
23	34	M	W	Nose and palate	Blastomycetes found in sections	Improved
24	35	M	C	Skin; systemic	At autopsy	Died. Autopsy showed blastomycosis of lung, heart, skin, bones
25	14	F	C	Skin; systemic	At autopsy	Died. Autopsy showed blastomycosis of lungs, liver, skin, and bones
26	55	M	C	Systemic; no demonstrable cutaneous lesions	Blastomycetes in sputum	Died. Autopsy showed generalized blastomycosis

morphologically and culturally were later identified as Blastomyces gilchristi. Roentgenographic study showed consolidation of both apices with large patches of bronchopneumonia in both upper lobes, together with destruction of the posterior ares of the third, fourth, and fifth ribs on the right side and erosion of the posterior ares of the fourth and fifth ribs on the left side. The tentative diagnosis was then changed to that of "Pulmonary Blastomycosis." The patient became weaker and died twenty days after admission. The anatomical diagnosis at necropsy was: *Generalized blastomycosis*, involving both lungs, spleen, kidneys, and posterior ares of third, fourth, and fifth ribs on the right side and fourth and fifth ribs on the left side; generalized arteriosclerosis; cardiac hypertrophy and dilatation; chronic valvular endocarditis; chronic fibrous myocarditis; areus senilis; emaciation. The following excerpts of the salient pathology are taken from the autopsy protocol:

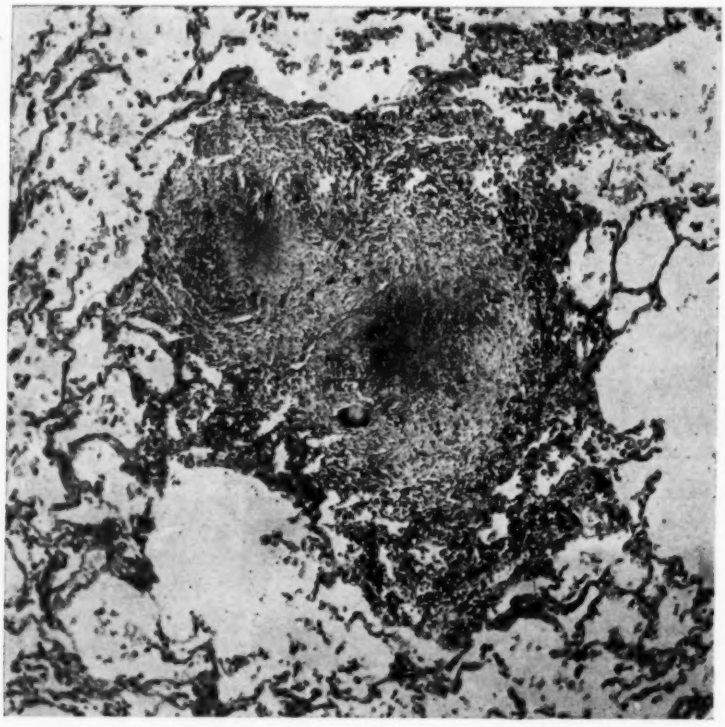

Fig. 1.—Tubercules in lung. No blastomycetes present.

Gross Observations—

Lungs: Both lungs present numerous discrete, elevated, button-like, grayish-yellow, resistant areas ranging in diameter from 4 mm. to 2 cm. The central parts of some of these areas are necrotic. Here and there areas of actual cavitation are noted. Those parts of the lungs that are air containing present the appearance of chronic passive congestion.

Spleen: Sectioned surfaces of the spleen reveal reddish-yellow, firm areas ranging in size from a pin point to 10 mm. in diameter, these being especially present in those parts subjacent to the capsule. The pulp scrapes away readily, and there is in addition, an apparent increase in the connective tissue element.

Liver: Is voluminous and bloody. Areas of focal necrosis are scattered throughout.

Microscopic Observations.—The puru-sanguinolent material secured from the bronchi and alveoli of both lungs as well as that from the eroded ribs shows the presence of a large number of yeast-like bodies both budding and nonbudding. In the *lungs* a variety of lesions are noted by microscopic observation and, as so admirably described by Irons and Graham,

LeCount and Myers, Medlar and numerous other investigators, the process is "generally bronchopneumonitis, and more a bronchitis than a pneumonia." The lesions, in general, are adjacent to the large blood vessels and bronchi, although some of the latter are difficult of recognition, many being entirely obliterated. The alveoli occurring in markedly affected areas are filled with fibrin and inflammatory cells. In numerous "nodular" areas typical tubercles are present, some showing caseation. Many bronchi show cellular detritus and occasional blastomycetes within their lumen with numerous mast cells infiltrating the bronchial wall. Although it may be said that generally the process is more highly cellular and less necrosis is present, still the lesions are indistinguishable from those of tuberculosis. Some

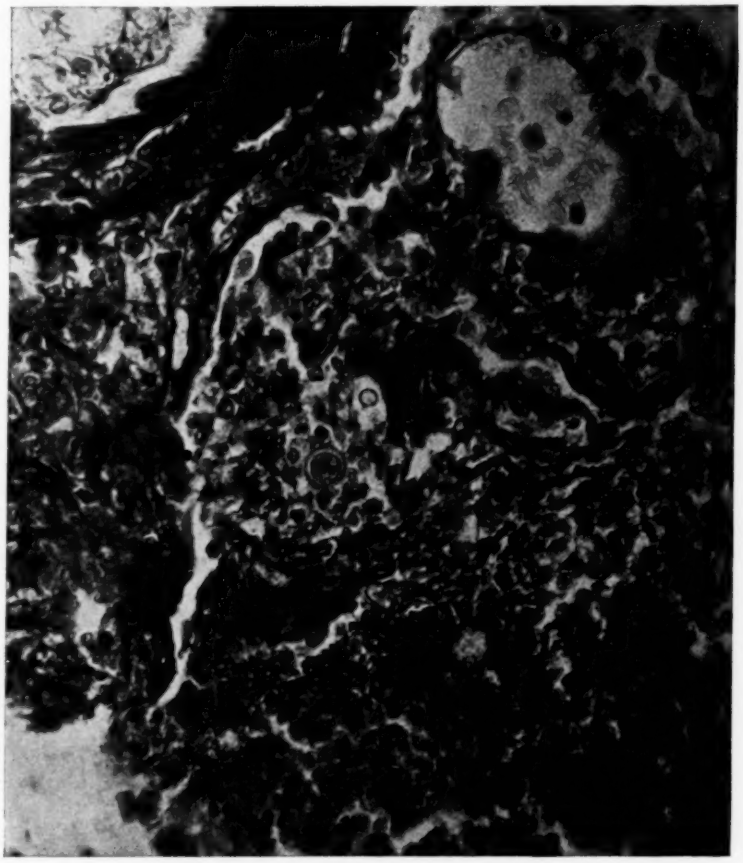

Fig. 2.—Same lesion as Fig. 1, different level showing causal organism.

typical mononuclear tubercles present at certain levels, no blastomycetes, while the identical lesions at different levels show many fungi. The giant cells differ in size and distribution. In some areas they are few in number, but in others they are very numerous. At times they show the inclusion of blastomycetes both active and dead, within their protoplasm, as many as 12 being counted in some. In least affected areas many alveoli contain desquamated epithelium and cellular detritus; some are emphysematous. In the *lymph glands* occurring about large bronchi, blastomycotic nodules are present. These usually show many blastomycetes within giant cells and free. Some of the nodules extend through the glandular capsule and then are usually limited by granulation tissue occurring about the bronchus. Some lesions show much caseation; others, little.

Bone: Portions of the left fifth rib, including its necrotic margin when decalcified

and prepared in the usual manner, show a fibrinous exudate in which bone, cellular detritus, and inflammatory cells are enmeshed, together with numerous blastomycetes, many budding.

Spleen: Numerous small focal lesions are present with necrosis not marked, but many blastomycetes present. In some sections there are present, irregular areas of fibrous tissue enmeshing a few giant cells, some showing inclusions of dead blastomycetes.

Liver: Minute focal lesions are numerous, usually occurring within the lobules. Necrosis is not prominent. Giant cells and fungi occurring free and within giant cells are numerous. Areas of fibrous tissue within which giant cells and blastomycetes are enmeshed occur.

Fig. 3.—Blastomycosis. Lung-Bielschowsky-Maresch-Foote Silver method.

DISCUSSION

In practically all reports dealing with the histopathology of infections caused by yeast-like bodies prominence is given the occurrence of lesions resembling those of tuberculosis. Gilchrist and Stokes state that in cutaneous blastomycosis the deeper portions of the chorion show tubercle-like nodules. Ricketts says that the nodules and giant cells of cutaneous oidiomycosis are indistinguishable from those of tuberculosis. Hektoen notes that coccidiodal granulomatosis mimics tuberculosis to an extent that the lesions cannot be differentiated microscopically. Stoddard and Cutler observe as a result of experimental work and study of clinical material that the differential diagnosis between tuberculosis and fungus infection would be impossible without finding the parasites. Davis found caseation and giant cells quite like tuber-

culosis in coccidiodal granuloma with tubercles which he thought not as clearly defined as those of tuberculosis. Medlar emphasizes the identity of the pathology in fungus and tuberculous infections, and states that it is only the presence of parasites in the pathologic lesions that establishes the type of infection. Miller shows that growth of the reticulum of the lung, and as he believes, its further transformation into collagenous tissue differs in no way in the tubercles in blastomycosis from that in the tubercles of tuberculosis, and concludes that if the presence of the causal factor be eliminated, the lesions of both infections cannot be differentiated. The striking similarity of the lesions both gross and microscopic in the two classes of infection is un-

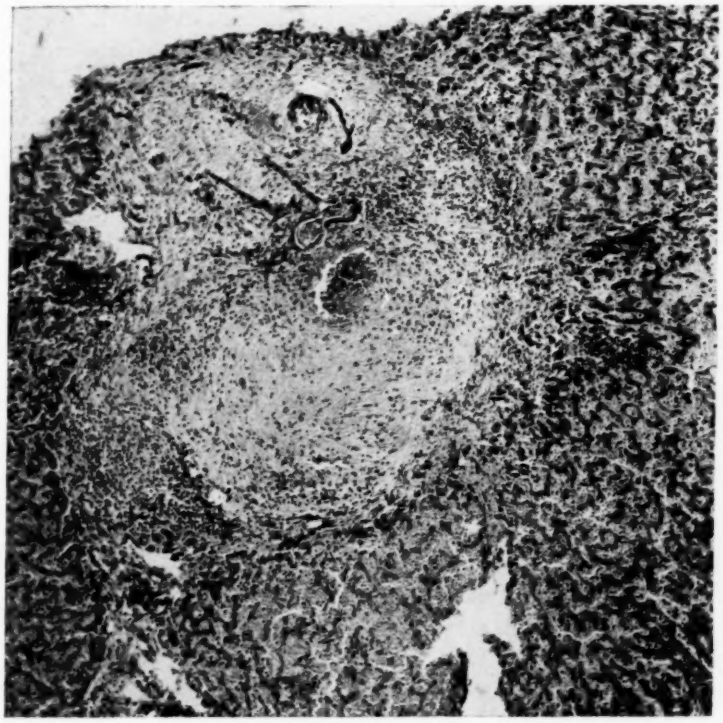

Fig. 4.—Blastomycosis liver—old healing lesion.

doubtedly outstanding. Still, such a similarity in lesions produced need not be surprising when we consider that new methods of investigation seem to indicate biologic relationship between the fungi and those microorganismal forms classed as acid fast.

Study of sections from the various organs from this case and others, after silver impregnation by the Bielschowsky's-Maresch method as modified by Foote, and staining with Mallory's Phospho-molybdic acid hematoxylin, leads us to agree with Miller, that silver impregnation allows a much better study of the causal organisms of blastomycosis than does staining with hematoxylin and eosin. With it, both active and dead forms of the fungus are readily demonstrable. The active organisms are generally spherical and markedly stippled. The dead forms appear either as uniform dark spheres

or clear-centered shell-like structures. These methods furthermore allow observation to be made of the reticulum of the various organs. Lesions in the lungs comparable to Miller's type I, II, III of tuberculous bronchopneumonia were readily demonstrable in a number of cases. In some tubercles in which caseation had not taken place, development of reticulum and the presence of collagenous tissue were readily observable. In caseous lesions, invading networks of reticulum rendered prominent by the silver, could be noted. Only the presence of the specific organisms either active or dead, allowed making an histologic differentiation from tuberculosis in any of the cases.

SUMMARY

The cases of systemic blastomycosis occurring at the Charity Hospital of New Orleans from 1906-1929 are recorded with detailed observations of the pathology in a typical case. Similarity of the gross and microscopic lesions in this condition with those present in tuberculous infections is again emphasized. This similarity necessitates demonstration of the causal agent before positive differential diagnosis can be made. Lesions in the lungs comparable to Miller's types I, II, III of tuberculous bronchopneumonia were encountered.

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THE RÔLE OF BUFFERS IN CHEMOTHERAPY*

By F. R. Greenbaum, D.Sc., and P. A. Kober, Ph.D., Chicago, Ill.

IN CHEMISTRY, the word "buffer" is ordinarily understood to mean any substance which maintains the hydrogen-ion concentration of a solution. However, in its original meaning and in its broader aspect "buffer" is defined, according to the *Encyclopaedia Britannica*, as a member which receives and absorbs the shock of an impact very gently and thus protects other parts from damage.

It is in this broader and original meaning of "buffer" that we employ the term in chemotherapy.

A chemotherapeutic agent often produces a shock or a systemic or local reaction that is due either to the high concentration necessary for its administration or to the chemical composition of the substance. This shock or reaction may be very mild or quite severe. At any rate, it is an undesirable occurrence which may prohibit the use of certain chemotherapeutic agents or make necessary special forms of administration, as in the case of arsphenamine and iodoxybenzoic acid. These have to be given in high dilution by the gravity method as they are too reactive to be given intravenously or intramuscularly in concentrated solutions.

Some of the papers published recently on buffers are: Biochemical Importance of Buffering Capacity;[1] Buffering and Alkali Reserve of Blood;[2] Intravenous Injection of Buffer Solution of No Value in Treatment of Inflammations;[3] Efficiency of Bicarbonate and Phosphate Buffers for Experimentation with Excised Organs;[4] Buffer Action of Saliva;[5] Buffer Action of Casein in Milk;[6] Buffer Capacities of Acacia and Tragacanth;[7] and many others. All of these rather recent publications deal with buffers from a point of view of hydrogen-ion concentration. In none of the above papers are buffers mentioned in the broad sense in which we are using the term. We use the word to mean any substance or group of substances which prevent or decrease the shock or reactions that certain chemotherapeutic agents, if administered alone, would produce. Besides this property of preventing shock, buffers often possess the property of stabilizing an otherwise unstable chemotherapeutic agent. Some of these buffers undoubtedly play a rôle also in maintaining a favorable hydrogen-ion concentration.

We shall attempt to illustrate the use of buffers in chemotherapy and point out how these substances buffer the various chemotherapeutic agents.

In the treatment of varicose veins, various chemicals have been tried. The treatment consists of injecting the varicosity with a chemical which

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causes coagulation of the blood within the vein and enough local irritation to the vessel walls to obliterate the lumen of the vessel through a local inflammatory reaction. The chemical must be injected in the offending vein and must be safe for intravenous injections. Sodium salicylate and sodium chloride have been used. Severe and prolonged reactions follow and if faultily injected, the result will be an intense local reaction in the infiltrated tissue which may lead to gangrene.

Dextrose in a 50 per cent solution will accomplish obliteration of a varicose vein but it is not as reliable as sodium chloride. However, it produces much milder reactions, without gangrene or sloughing. It is, therefore, safer than solutions of other drugs.

Kern and Angle,[8] after a thorough research, arrived at the conclusion that a combination of a 50 per cent solution of dextrose and a 30 per cent solution of sodium chloride is the best and safest preparation. This solution combines the positive and reliable action of sodium chloride with the buffer action of dextrose. This is an instructive illustration of the buffer action of dextrose on the sodium chloride which is used for the treatment of varicose veins. The dextrose, as mentioned above, may itself be used as a chemotherapeutic agent for varicose veins. Its action is mild but the combined use of sodium chloride and dextrose has the advantage that the dextrose prevents or decreases the chemical shock produced in the surrounding tissues by the sodium chloride. Just what the mechanism of this buffer action is, remains unknown.

Another illustration of a buffer action in chemotherapy is the use of a buffer with gold sodium thiosulphate which was introduced by Mølgaard for the treatment of pulmonary tuberculosis. This compound did not, however, live up to the claims of Mølgaard and today it is used almost exclusively in the treatment of lupus erythematosus.

Gold sodium thiosulphate is described in the literature as a white crystalline salt. The solid powder is stable in a tightly stoppered container. It is readily soluble in water but is unstable in an ordinary solution. This is the reason that gold sodium thiosulphate has, heretofore, been available on the market in solid form only. In our laboratories we have succeeded in perfecting a solution which will remain stable and perfectly clear for an indefinite period of time. This goal was reached by using sodium thiosulphate as a buffer. In this case the sodium thiosulphate acts as buffer against decomposition of the gold sodium thiosulphate and, due to some chemical action, prevents any photo-chemical reduction of the gold sodium thiosulphate. The sodium thiosulphate in this case acts as stabilizer for the otherwise unstable solution of gold sodium thiosulphate. This is an illustration where a buffer can also act as a chemical stabilizer.

Another excellent example of buffer action is the following: When the bismuth therapy was introduced by Levaditi, it was found that aqueous solutions of bismuth compounds, particularly the tartrates, caused considerable pain on injection. For this reason, the clinical use of water-soluble bismuth compounds was abandoned and oily emulsions were introduced and used almost exclusively. There are many objections to the use of oily emulsions. The main disadvantages are the unreliable and uncontrollable absorption and

the formation in the injected tissues of oil tumors or oil nodules so that, after a number of injections, little or no space is left for further injections. Another disadvantage is the danger of embolism should some of the material get into a vein. From a chemical point of view, there is also the possibility of bismuth soap formation on long standing (either in the ampul or in the tissues) due to hydrolysis of both the oil and the bismuth compound. In our laboratories, one of us (Kober[9]) elaborated a water-soluble sodium tetra-bismuth tartrate, containing 76 per cent of bismuth, which is readily soluble in water and which can be injected intramuscularly with very little pain. To overcome this pain, the aqueous solution of this sodium tetra-bismuth tartrate was buffered with a strong solution of sucrose. The sucrose acts as a sort of stabilizer, preventing the precipitation of the tartrate bismuth base through lactic acid production. Furthermore, the sucrose buffers the painful action of the tartrates and, by its endosmosis, increases the speed of diffusion and thus effects a prompt absorption.

A typical illustration of a buffer action which considerably decreases the toxicity of a compound may be found in the case of calcium salts, such as the chloride or the iodide. Calcium chloride, if administered intravenously, has a very high toxicity and only small doses can be given. If, however, a loose addition compound of calcium chloride with urea is used,[10] the toxicity is considerably decreased and much larger doses can be given than if calcium chloride is used alone. Thiourea and substituted ureas have a similar buffer action, forming loose addition compounds with either calcium chloride or calcium iodide as was shown by one of us.[11] There is no question but that, in these loose addition compounds, the urea and thiourea and their substituted derivatives act as buffers which permit larger doses of calcium chloride and calcium iodide to be injected intravenously.

And, finally, we would like to mention the use of dextrose as a buffer in combination with arsphenamine. H. Weitgasser[12] and others have used it in asthenic patients and in individuals who were intolerant to arsphenamine. They dissolved the arsphenamine in 20 c.c. of a 50 per cent dextrose solution. The dextrose decreased the toxicity of the arsphenamine and prevented a great deal of the shock produced by the arsphenamine alone.

Attempts to reduce the toxicity of arsphenamine were carried out by J. Oliver, S. S. Jamada and F. Kolos[13] who assert that the combination of arsphenamine with hydrophyl colloids reduces the toxicity of the drug. Of the colloids examined gelatin is the most effective. The authors point out that the decrease in toxicity is generally shown by the facts that the physical toxicity of arsphenamine is removed, the chemical toxicity is reduced to three-fifths. The circulatory disturbance following its administration is markedly lessened. This is undoubtedly a buffer action and gelatin as well as dextrose or any other food substance containing the active hydroxyl or amino group could be used.

I. B. Lipskeroff and F. N. Grebin[14] studied the comparative value of methods to reduce harmful effects of neoarsphenamine by different vehicles. They offer the following conclusions: Neoarsphenamine is used in vehicles other than water, so that it may be given in larger doses and at shorter in-

tervals. Of all vehicles used dextrose is the most promising. Gelatin, blood serum and calcium chloride have some effect in some cases.

Recently, Macht[15] showed that injections of mercurochrome in combination with 50 per cent dextrose were much less toxic than mercurochrome alone. Macht showed in animal experiments as well as in experiments on human beings that mercurochrome combined with 50 per cent dextrose is considerably less toxic than mercurochrome alone. In collaboration with several naval physicians several hundred cases were treated with this combination of mercurochrome and dextrose and this proved superior to mercurochrome alone. He found, however, that the dextrose on standing with mercurochrome, exercises a reducing action on the mercurochrome and a small amount of metallic mercury was thrown out. He therefore recommends to prepare this mixture of mercurochrome and dextrose fresh each time before injection. This reducing action of dextrose on mercurochrome is probably the reason that since Macht's discovery no other report on the use of dextrose and mercurochrome in combination seems to have appeared. However the action, aside from the chemical reduction, is undoubtedly a buffer action of the dextrose on the mercurochrome.

Dextrose is also used as a buffer in the intravenous injection of ethyl alcohol for general anesthesia.[16] Numerous other examples and illustrations of buffer action in chemotherapy might be cited but space and time will not permit an enumeration of them all. We hope, however, that through these few illustrations, we have shown that the possibilities for the use of buffers have by no means been exhausted and that various chemotherapeutic agents may be improved by the use of suitable buffers.

DISCUSSION

In the oral use of chemotherapeutic agents, high dilutions and buffers have long been used to avoid disagreeable taste and to diminish irritation in the gastrointestinal tract. In intravenous and intramuscular administration, the use of buffers is comparatively new. It is reasonable to believe that suitable buffers may be as necessary as the chemotherapeutic agents themselves. The agents, if at all potent, must have, in the concentration necessary for injection, considerable activity both locally and systemically before they are diluted by the blood and other body fluids. Suitable buffers would permit such active substances to be injected and allow chemotherapeusis without untoward reactions local or systemic. It is probable that a number of therapeutic agents, heretofore too irritating or toxic to be available for therapeutic use, might now be utilized if suitable buffers were found.

Some of the properties of buffers may be briefly mentioned:

1. They must be nontoxic and nonirritating themselves. Since the amount of buffer used is large, there are few substances that fulfill this qualification except foodstuffs or derivatives of foodstuffs.

2. They must contain either amino or hydroxyl groups, the two active biologic groups, which undoubtedly owe their buffer action to secondary valences.

3. They must be readily soluble in water.

4. They must be easily available and low in price so that the cost of administering the therapeutic agent is thereby not increased prohibitively.

Buffers containing hydroxyl groups are easily available in the form of sugars and other substances but amino derivatives, with the exception of urea, are as yet too expensive for this purpose. A cheap source of amino acids might give us a considerable number of useful buffers.

SUMMARY

Summarizing, we can say that the term "buffer," used in its broader meaning, not only refers to a substance which lessens the activity of acids or alkalies but includes such substances as decrease and delay the shock of an action or reaction and thus protect other parts from sudden change or damage. In this sense we use the term "buffer" in chemotherapy.

Chemotherapeutic agents often produce a shock or a reaction because of the high concentration necessary for their administration. In many cases this shock can be avoided and the reaction decreased or prevented by the simultaneous administration of substances which will buffer this action. Often, the buffer action is mainly that of a stabilizer.

As typical illustrations, the following were discussed:

1. The use of dextrose as a buffer in sodium chloride, dextrose solutions for varicose veins. The use of dextrose (glucose) with arsphenamine, mercurochrome and ethyl alcohol solutions were also mentioned.

2. The use of sodium thiosulphate as a buffer in gold sodium thiosulphate solutions.

3. The use of sucrose in bismuth sodium tartrate solution, the sucrose acting as a buffer.

4. The use of urea and thiourea with calcium chloride and calcium iodide to decrease the toxicity of these compounds.

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POSTINFLUENZAL SINUS-NODAL BLOCK*

A Rare Disturbance of the Heart Following an Infectious Disease

By Aaron E. Parsonnet, M.D., F.A.C.P., Newark, N. J.

WITHIN recent years, considerable attention has been drawn to the cardiovascular changes following acute infectious diseases. Such minor conditions as the simple respiratory tract affections have been discovered to leave unmistakable traces upon the heart and blood vessels. The ordinary dismissal of such infections as being rather benign, must in the light of recent investigations be considered as unscientific and therefore unsafe.

Modern medical literature is replete with reports attesting to the serious myocardial damage that may result subsequent to even the mildest clinical manifestations of the infectious diseases. Most of the authors lay particular stress upon the fact that irreparable damage to the heart may occur unless the condition is discovered early through careful examination of the patient's cardiovascular system. Most of the clinicians also agree in suggesting the need of electrocardiographic control in every case showing the slightest cardiovascular impairment. Many types of cardiac arrhythmias have been described and mention need be made only of first degree of heart-block which is especially common in nearly every acute infection. Second and third degree of heart-block, while less common, has been described by Hyman[1] in a study of delayed conduction in influenza.

The available literature, however, yields no demonstration of an uncommon form of heart-block known as "sinoauricular block," following any type of infectious disease. The following case is therefore of interest:

CASE REPORT

P. H., a young Polish laborer, twenty-nine years of age, with a previous negative medical history, had suffered what he called a "slight cold in the head." He had the typical symptoms of an upper respiratory tract affection with a reddened throat and slight elevation of temperature. The acute symptoms lasted only a few days but he felt very much "run down" and upon returning to his usual work of wire-making, found that he tired very easily and was unable to keep up with the pace set by his fellow laborers. For the first time in his life he experienced a peculiar sensation of oppression and breathlessness referable to the precordium and which is probably described best under the term, "heart consciousness."

The plant physician noted an irregularity in the pulse and sent him to me for detailed cardiovascular study.

The patient was a well-built young man weighing 130 pounds, and apparently in good health. The general physical examination, with the exception of the cardiovascular system, was negative. The heart was somewhat enlarged, the heart sounds were clearly heard over the entire precordium but were of rather poor "tonal quality." No murmurs or extra-

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cardiac sounds were elicited. The pulse was rapid, ranging from 110 to 134 beats per minute. A definite irregularity, similar clinically to a "dropped beat," was heard and felt about six to eight times a minute; this irregularity remained unchanged after exercise.

Electrocardiographic study demonstrated that the irregularity was not due to extrasystole but to a rather unusual arrhythmia described as sinoauricular block. (See Figs. 1 and 2.)

It will be noted that there is a true intermission in the cardiac cycle which occurs about every sixth to ninth beat. The intermission takes the form of a complete dropping out of an entire cardiac cycle. The next normal beat occurs at the time of the dominant rhythm, there being no alteration in it; in other words, a cardiac cycle is dropped from the rhythm. This condition is said to occur whenever the pacemaker in the sinus node fails to deliver an impulse for cardiac contraction. The rather awkward use of the term "block" refers to the conception of some interference in the origin of the stimulus.

The tracings also show other changes suggestive of some nodal and auricular disease. It will be noted that the P-waves are normally upright in Lead I, but they are inverted in Leads II and III. The P-wave following the intermission caused by the dropped cycle is, however, upright in these two leads suggesting that there is a recovery phenomenon in this condition.

There is much reason to believe that the condition, perhaps, may be due to a faulty development of the stimulus in the pacemaker cells and that it may be produced not so much by a blocking of the stimulus as through its suppression. The latter view would tend to explain other disturbances of the pacemaker of which this is one. Many theories have been evolved to explain the process which so affects the generation and release of the stimulus for the orderly sequence of events required by the normal cardiac cycle. Two schools of thought have arisen, one maintaining that the threshold of resistance through which the impulse must pass from its origin in the sinus nodal cells to the adjacent auricular musculature is increased to such an extent that the excitation wave finds difficulty in emerging. This increased threshold of resistance may be the result of vagal stimulation or it may be associated with toxic effects during the course of an acute infectious disease. Some authors like Levine[2] and Hewlett,[3] believe that digitalis may exhibit the same physiologic type of blocking at the sinus node as is commonly demonstrated in the auriculoventricular node. Mackenzie and Schott have shown that this condition may arise as a toxic depression of the myocardium; influenza and acute rheumatic fever may so modify the course of the excitatory process that more or less complete obstruction to the passage of the impulse may occur. This latter condition has been demonstrated by many investigators; the prolongation of the P-R interval in the electrocardiogram is frequently seen during the course of acute infectious disease. This prolongation is assumed to be due to a retardation of the impulse through the auricular muscle from the time that it leaves the sinus node until it reaches the lower node. When the same process occurs at the threshold of the sinus node the impulse may be entirely blocked.

The other view held by such able investigators as Wenckebach[4] and Resnick,[5] is that the condition arises not because of any increase in the

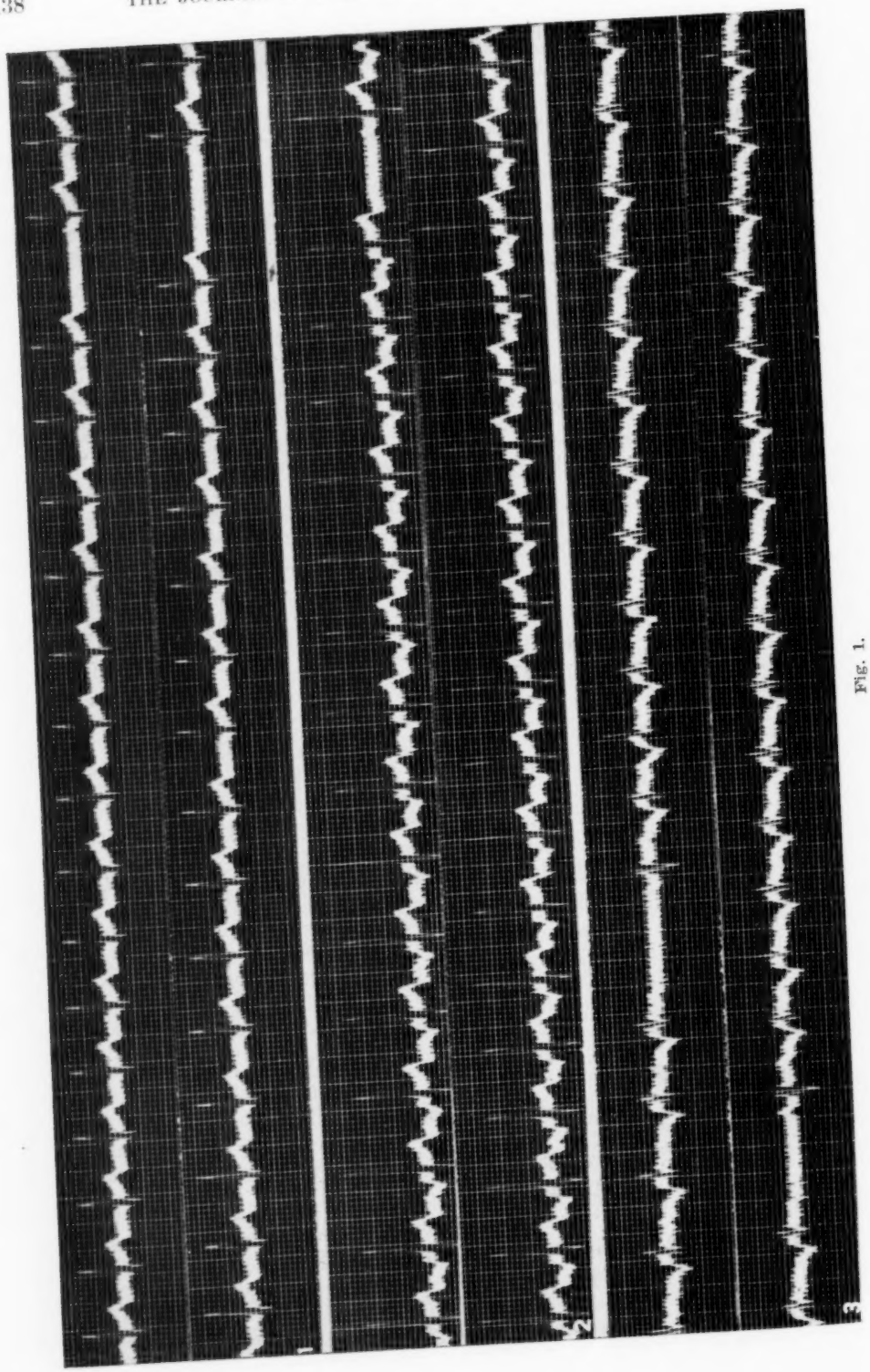

Fig. 1.

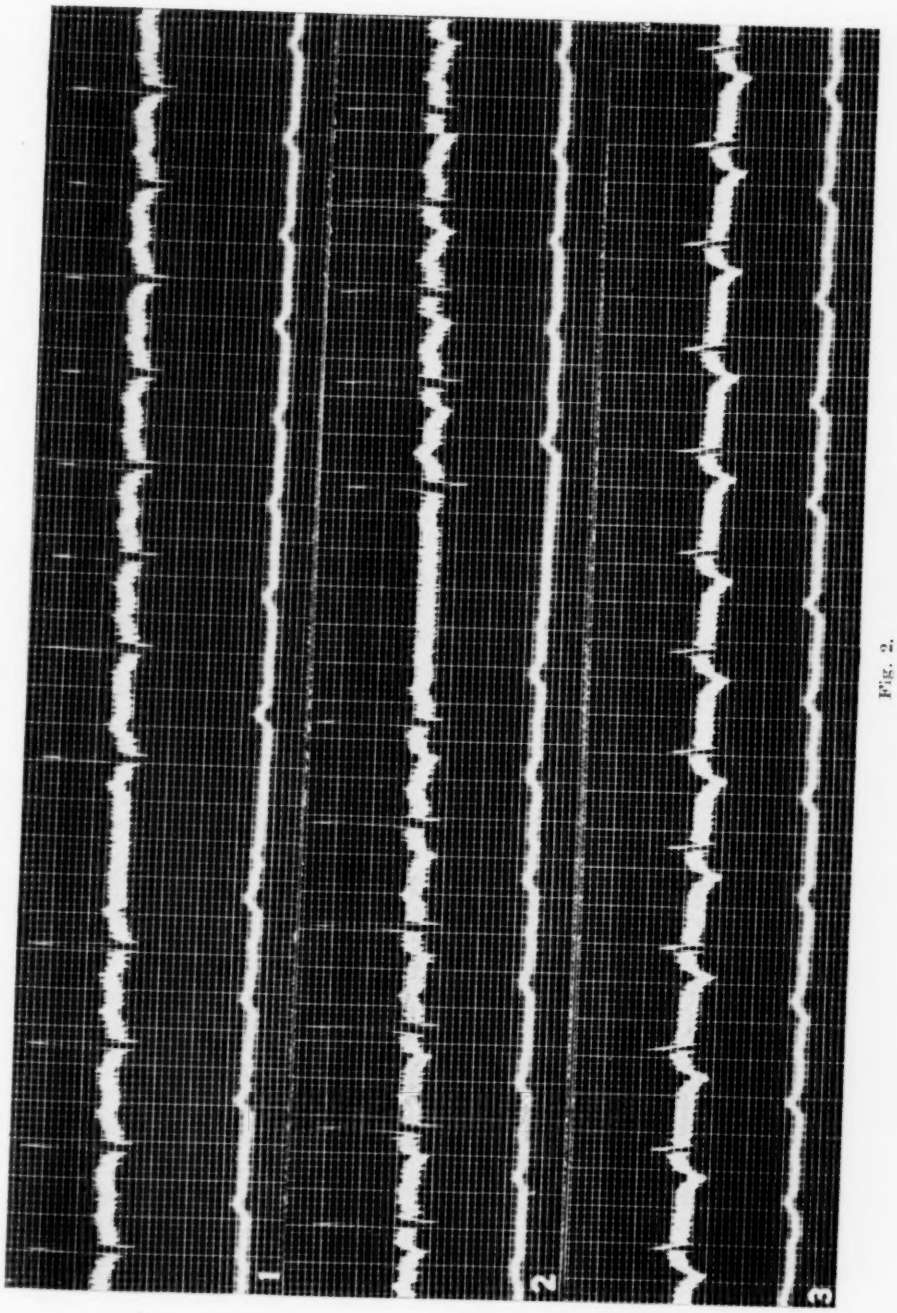

FIG. 2.

threshold value of the adjacent myocardium, but because the generative function of the sinus nodal cells is lessened. This may result either from decreased metabolic intercellular activity of the sinus node or because of the imperfectly developed vagotonic changes.

Regardless of the theoretical background for the ineffective development of the stimulus, the ultimate result is failure of regular release of the excitatory process to spread in its usual pathway from the sinus node to the node of Tawara.

Clinically, this is indicated by an irregularity in the heart and pulse-rate similar in respects to an A-V block or to the untrained as an extrasystolic arrhythmia. Indeed, without graphic assistance its recognition may be impossible. Electrocardiographic tracings of the condition show the complete dropping out of a cardiac cycle; theoretically, the pause should be equal to two normal beats, but more often this interval is either slightly shortened or lengthened. In the record presented here both conditions are present. It was the variability in this interval which led Resnick to advance the theory that sinoauricular block was merely a manifestation of an exaggerated sinus arrhythmia and that, indeed, "sinoauricular standstill" cannot be regarded as a special entity.

This case is interesting in pointing out that sinoauricular block may occur after acute influenza or any other upper respiratory tract infection. The relative rarity of this condition makes it one of especial speculative interest. So far as prognosis is concerned, the condition itself can only be taken as evidence of the toxic reaction of the heart to the acute infectious diseases. The heart muscle, the conducting system, the valvular mechanism and the pericardium, have all been shown to be seriously affected by these infections; the sinus node itself is apparently not immune to such toxic disturbances.

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THREE MADISON AVENUE.

STUDIES IN THE ALIMENTARY TRACT OF MAN*

VII. The Gastric Responses to Water and Lactic Acid

By Wilhelmine Kuenzel, A.M., and T. Wingate Todd, F.R.C.S. (Eng.), Cleveland, O.

INTRODUCTION

IN OUR previous articles we have demonstrated the necessity of using trained and stabilized stomachs for the investigation of gastric response in Man.[1, 2] The effect of interferences of central origin is marked but it can be minimized or even eliminated.[5, 8] Upon carefully stabilized stomachs we have been able to show the differences in gastric response to milk and to buttermilk.[3] Heat and cold when introduced into the experiment, whether directly to gastric mucosa or indirectly through abdominal wall, have a distinct effect upon the behavior pattern.[4] Indeed there is evidence that the reflex effect of these stimuli applied through the abdominal wall is more pronounced than that produced by direct stimulation of mucosa.

In the present paper we deal with the behavior patterns of water and of lactic acid. Warned by our previous experiments, we are careful to keep the temperature of the vehicle constant at 70° F.

General conditions of the experiment such as size of meal, time of day, control of previous eating, drinking and smoking are standardized and have already been discussed. Consequently there remains only to survey briefly the reaction patterns of milk and buttermilk which act as contrasts for the water and lactic patterns about to be described.

A milk meal results in a shadow area of smaller dimensions than that following buttermilk. (Fig. 1.) The difference in shadow length is less than that in breadth; initial lateral distention is a marked feature of the buttermilk stomach.

Peristaltic activity is much more vigorous in the buttermilk stomach. Gastric activity after milk may take the form of shallow peristalsis, frequently visible over the pyloric part only, or of an ill-defined and apparently purposeless shimmer. Buttermilk peristalsis is of considerable amplitude, obvious on both curvatures, constricting the lumen even to subdivision of barium shadow, seen often as high as the Magenblase, sometimes accompanied but never replaced by shimmer.

Passage of contents through the pylorus, after a milk meal, is copious enough but appears as a light shadow like a puff of smoke. The buttermilk

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shadow, on the contrary, is dense black and therefore looks more voluminous. In the erect posture in which all our gastric roentgenograms are taken, the duodenal cap frequently appears early and full after buttermilk but late and imperfect after milk.

These then are the more obvious characteristics of the milk and buttermilk patterns. They serve as our background for the study of other responses. Since the buttermilk used is merely a lactic milk, it is obvious that, in our further analysis, we should know whether the specific phenomena of the

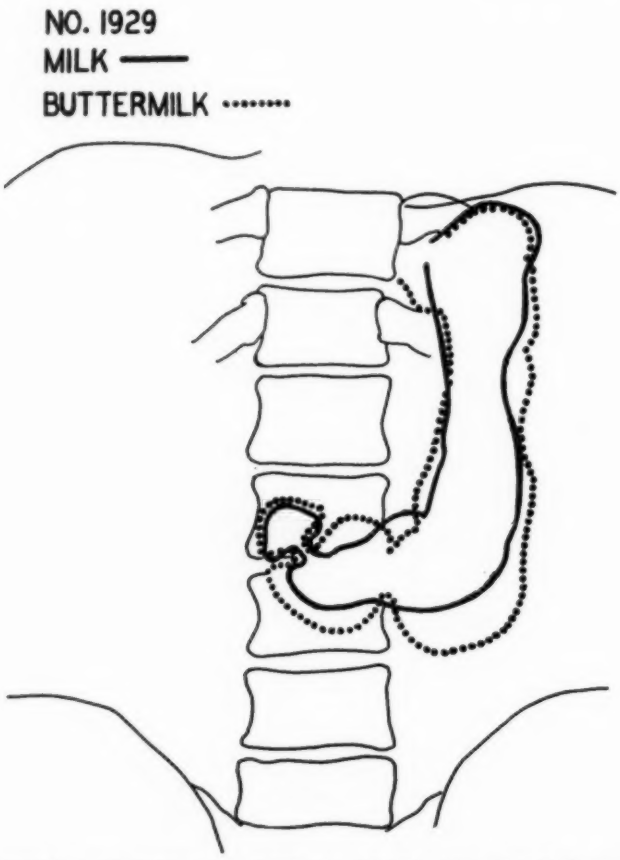

Fig. 1.—Comparison of stomach No. 1929, ten minutes after standard meals of milk and buttermilk. The milk stomach shows less vigor, a smaller shadow area and shorter length. The difference in breadth in this stomach is less than usual.

buttermilk pattern can be duplicated by lactic acid. This first necessitated an investigation of the water pattern as a basis for the lactic study. Of this inquiry one aspect only has been completed, namely, the contrast between milk and buttermilk patterns.[3] There remain for consideration the comparison of milk and water patterns, of buttermilk and lactic patterns, and the contrast between water and lactic patterns. As a general introduction to these successive studies we must first describe the conditions of experimentation.

CONDITIONS OF EXPERIMENTATION

The observations were made on sophomores in October, 1928. The meal consisted of 33 gm. of barium sulphate in 4 ounces of vehicle, a total of 5 ounces. It was swallowed about two hours after breakfast, the subject having neither smoked nor taken a drink of water in the interval. Temperature of the meal was kept constant at 70° F.

The examination of each stomach in these studies continued over two days. On the first day the vehicles used were milk and buttermilk; on the second, water and lactic acid. Four students were examined at each session. On the first day two received milk first and buttermilk an hour later, the other two took the buttermilk an hour before the milk. On the second day a similar alternation was arranged for water and lactic acid. It is our experience that the characteristic gastric response to a particular stimulus is more pronounced in a stomach already recently stimulated (Facilitation[3]). Hence the alternation of order in administration of meals.

To ensure adequate comparison the acidity of our lactic meal was maintained at pH_4, the approximate acidity of buttermilk. A number of preliminary experiments were necessary to determine the best method of administration. In this we had the advantage of consulting Dr. T. Sollmann and Dr. V. C. Myers to both of whom our indebtedness is gratefully acknowledged. In the course of our studies we have drawn repeatedly upon Dr. Sollmann's expert knowledge and breadth of scientific understanding. And while we would not saddle upon him our interpretations or conclusions, it gives us great happiness to express our obligation. To Dr. E. Muntwyler also our grateful thanks are due for providing us with the various solutions required.

The phenomenon of facilitation and the four-phase subdivision of our study require careful planning in the order of administration of the various meals. This is expressed in the following tabulation:

Series I (8 students) Milk, buttermilk, water, lactic acid.
Series II (6 students) Buttermilk, milk, water, lactic acid.
Series III (7 students) Milk, buttermilk, lactic acid, water.
Series IV (7 students) Buttermilk, milk, lactic acid, water.

THE PRELIMINARY STUDY

A preliminary study to determine the most advantageous conditions for the experiments was made upon our two standard experimental stomachs regularly used for this purpose. We fed to them 5-ounce opaque meals of water and of lactic acid solution. The water experiment required no further preparation, but the possible effect of differences in acidity of the lactic solution needed clarification. Dr. Muntwyler kindly prepared for us three solutions of different acidity. No. 1 was buffered with sodium hydroxide to a pH of between 2 and 3. No. 2 had a titrated acidity equal to that of buttermilk. No. 3 was again buffered with sodium hydroxide but this time to pH_5. Solution No. 1 is more and No. 3 is less acid than buttermilk itself.

Thus we were able to test the effect of different acidities measured by hydrogen-ion concentration, and also to compare the effects of titrated and

free ion acidities. Our two test stomachs gave entirely comparable results. In each the water pattern was the most active. Of the lactic solutions, No. 3 of pH_5 induced the greatest activity. To our surprise lactic solution No. 1 of pH_{2-3}, produced a gastric pattern of little vigor, and the stomach shadow showed greater dimensions than after either of the other lactic solutions or after water. It is obvious from our previous investigations[5, 8] that central nervous impulses affect the gastric response and both subjects expressed marked distaste for lactic solution No. 1. Solutions No. 2 and 3 were not unpalatable to either subject but a slight preference was expressed for No. 3.

As a result of this experiment we decided to use a lactic solution of equal acidity with buttermilk determined by hydrogen-ion concentration and buffered with sodium hydroxide to simulate the buffering in buttermilk.

DIMENSIONS OF GASTRIC SHADOW

In order to obtain all possible information from the roentgenographic shadow the vertical height, width at base of Magenblase, and area of shadow have been measured upon the series of sophomores participating in this study. The methods of measurement have been presented in our earlier papers[1, 2] and the results are given in Table I. Of the twenty-eight stomachs only those to which the meal was administered second can be utilized for comparison because in them the impulses of central origin have been minimized, and the characteristic features of outline intensified. This theme has been discussed previously[3, 4] under the caption Facilitation.

Since the Magenblase itself varies considerably in height and volume, it follows that the base of the Magenblase is inconstant in position. Therefore the average gastric shadow widths set forth in the table are unreliable and cannot be considered as measures of the gastric reaction to various barium vehicles. Nor indeed can this particular width, measured upon the same stomach under different stimuli, be a dependable dimension.

Turning next to vertical height we observe, from the table, that the milk and water averages are the same, that the lactic acid average is greater

TABLE I

	MILK	BUTTERMILK	WATER	LACTIC ACID
No. of stomachs	24	25	26	25
Meal given first	10	12	13	12
Meal given second	14	13	13	13
Area sq. mm.				
Total average	10530	11832	10458	10627
First meal average	11509	9993	11632	10409
Second meal average	9831	13683	9284	10828
Vertical height in mm.				
Total average	203	204	194	199
First meal average	221	185	202	195
Second meal average	185	223	186	202
Width at Magenblase base in mm.				
Total average	48	50	51	50
First meal average	50	51	54	51
Second meal average	46	49	48	50

and that the buttermilk average easily outdistances all others. Since some stomachs elongate obliquely, this does not give an accurate measure of elongation but merely indicates that there is a greater extension in stomach length after buttermilk and lactic acid than after milk or water.

Despite our inability to use gastric width and our retention of vertical height as an index rather than a measure of elongation, we do find the third estimate of dimension, namely shadow area, of significance. In our experience of this series and also of others in which we have had the opportunity to compare gastric dimensions, we feel certain that the water shadow is as small as and often smaller than the milk shadow. We are also convinced that the lactic acid shadow is usually larger and the buttermilk shadow much larger than the milk. These convictions are expressed in the average shadow areas of the second meals. Bearing in mind the possible error of 7 per cent on planimetric records[1] the average figures are equivocal only for the relation in shadow area of milk and water.

It must be understood that the size of shadow does not of itself indicate definitely its causation. We are not dealing here with differences in "tone" of the stomach as in our contrast of shadow area in freshmen and sophomores. The present comparison involves trained stomachs which have been further stabilized by administration of an earlier meal under the same circumstances the same day. It is true that shadow dimensions bear some, as yet undefined, relation to volume, but stomach volume is by no means simple in its interpretation. The size of the shadow is increased by secretion of gastric juice and diminished by an open pylorus. Hence the stomach volume really depends upon the relation between rate of secretion and rate of emptying. Finding, as we have stated, the water stomach relatively small in its dimensions, we were at first inclined to believe that after a drink of water there was comparatively little secretion but an almost continuously patent pylorus. This seemed to be confirmed by the rapid emptying of the water stomach. It was expressed in a recent abstract of our observations by the senior author.[6] This however is an error in interpretation. We have repeatedly watched the large flow of fluid through the pylorus in water stomachs, so large indeed that it could be accounted for only by a profuse secretion of gastric juice.

In summary we should state that, in our experience, the buttermilk stomach is most laterally distended for a given size of meal. Somewhat narrower is the milk stomach. The lactic stomach is intermediate in width between the milk and the water which is certainly the narrowest. We have not been able to indicate this relationship quantitatively owing to the difficulties enumerated above.

The buttermilk stomach is the longest and has the largest shadow area. Next to this comes lactic acid, then milk, and smallest of all water. We have been able to present these findngs in Table I. The qualified success of our efforts is due to the number of factors which influence the shadow. As the work progresses we become better acquainted with these influences and a full presentation of our observations will follow in due time.

THE WATER PATTERN

If one of our standard five-ounce water meals ($Ba\ So_4$ 33 gm., water 4 oz.) be administered to a stabilized stomach without facilitation by a previous barium meal, there seem to be set up immediately waves which course over the entire stomach from upper gastric tube to pylorus with very little effort. The waves are not purposeless but they give no impression of force in spite of the fact that their amplitude increases during the first two minutes or thereabouts. After some ten minutes the amplitude is again reduced and no further change is to be seen during the hour after taking the meal.

This immediate setting up of a wave pattern is an illusion. Provided there be barium in the stomach from a previous meal, one may readily observe that the administration of water merely slightly deepens the waves already passing over the greater curvature. We have not previously emphasized these waves which occur in the stomach between meals. They are usually known as hunger contractions. We find it difficult to call them by this name since they are always present in the stomachs examined by us unless inhibited by emotional disturbance. In a previous paper we have spoken of them as the waves of the neutral phase apparent in the stomach twenty minutes after a five-ounce opaque meal.[4] When the stomach is in neutral phase, neither the nature of the contents nor the character or temperature of the meal can be discerned.

In view of the observations just recorded it is clear that the administration of water has not inhibited the gastric waves but slightly increases their amplitude during the first two minutes. When, after about ten minutes, the amplitude is reduced, it merely means that the neutral phase once again supervenes and all influence of the water has ceased. This speedy return to the neutral phase calls for further explanation. As water is a powerful stimulant of gastric juice it follows that the stomach is well flushed after a drink of water. Since the shadow of a water stomach remains relatively small, the pylorus must be opened either more frequently or for longer periods than after other meals. Nevertheless we do not see the passage of water contents in that massiveness which is so evident after buttermilk. The explanation of this lies in the fact that while barium is easily suspended in buttermilk it readily settles in water and therefore is less obvious in passage. Assiduous observation of the pylorus after a water meal however demonstrates to one's entire satisfaction the almost continuous passage of much fluid with a mere wash of barium in it.

SIGNIFICANCE OF THE WATER PATTERN IN GASTRIC STUDY

This behavior pattern of the water stomach throws considerable light upon the actual events in the stomach and their interpretation. Between meals the neutral waves (hunger contractions), of moderate amplitude and considerable speed, course over the gastric walls in fairly rapid succession. These gastric waves are not inhibited by water but deepened somewhat without increase of vigor. Within ten minutes the effect of the small water meal has passed and the waves once again have assumed their neutral character.

The barium which has settled out of the water along the greater curvature remains in large part as a precipitate, washed by constant flushing of gastric juice. One may ask if this barium is responsible for the neutral waves which are so obviously coursing over it. We can observe the same waves in stomachs to the mucosa of which only the tiniest vestiges of barium cling. It seems absurd to claim that so small a dose of an insoluble salt could produce so definite a result. In certain thin individuals we have been able to follow with ease the course of the neutral waves along the greater curvature without the use of barium or other roentgenoscopically opaque substance. As for the constancy of the neutral wave rhythm we have no conclusive evidence. It has invariably been present in the stomachs we have examined except in the presence of some definitely ascertainable emotional disturbance. Carlson and his co-workers[7] describe interruptions of the rhythm unrelated to such a cause. This is not definitely contradicted by our experience which, in this study, covers some 500 stomachs, and it is possible that the difference in viewpoint is merely one of interpretation.

The waves of the neutral phase can be seen in suitable patients even in the gastric tube where no barium remains to outline their pattern. They pass over the entire stomach from Magenblase (fundus) throughout gastric tube, pyloric vestibule and canal to pylorus. A drink of water at 70° F. modifies neither their range nor speed but slightly increases their depth. In our opinion it is a mistake to differentiate between hunger contractions and digestive waves. Gastric peristalsis is modified under certain conditions. The basic pattern is the neutral phase rhythm. This is but slightly modified by a small amount of water. The further modifications by milk or other vehicles and by heat or cold will be classified after we have completed the description of our experimental work. We must, however, at this stage, make clear our interpretation of gastric motility.

We have already referred to the small gastric shadow and almost constant passage of fluid through the pylorus after a 5-ounce water meal. We have also mentioned the manner in which the barium sinks to the greater curvature and is but slowly washed away through the pylorus. The rapid return to neutral phase rhythm makes a small water meal the best preparation for a roentgenoscopic study. It provides a washed precipitate of barium as a delineator of gastric outline. Its particular influence on gastric motility quickly passes away, leaving apparent the neutral phase rhythm which can then be made the basis for judging the effect of some other modifying influence such as buttermilk or heat.

"COGWHEEL" OUTLINE AND "BALLOONING"

The relatively rapid elimination of the water influence seems definitely connected with profuse gastric secretion which brings about no increase in gastric shadow because the pylorus is almost continuously patent. If anything, the tone of the gastric tube is increased in the water stomach. Certainly the rugae often stand out upon the roentgenoscopic picture of the gas-

tric tube like cogs. We have not seen this feature in our other studies. The suggestion is raised that perhaps passage onward of liquid gastric contents to the duodenum is more a result of flushing than of peristalsis.

Another feature of the water pattern, occurring erratically and by no means present in every individual examined, is the "ballooning" of the gastric tube by gas from the Magenblase. In our discussion of shadow features we have referred to the inconstancy in floor level of the Magenblase. At certain moments the Magenblase extends in pyriform shape down the gastric tube. If the stomach be an active one, as it is in the water pattern, this pyriform outline becomes moniliform. Thus the gastric tube appears "ballooned" with gas. The picture, though recurrent, is naturally quite temporary and has been observed by us most frequently in water stomachs, quite often however in lactic acid patterns and very rarely, even in its pyriform phase, in other gastric behavior studies. Again we cannot help connecting this "ballooning" with the rapid passage for it appears as though the gas in the Magenblase were drawn down the gastric tube by the siphon-action of the column of liquid. The phenomenon must be further studied.

WATER DRINKING AS A MANIFESTATION OF NERVOUSNESS

While discussing the water behavior pattern it is necessary to refer to a phenomenon which caused us great confusion in the earlier part of this study. Very frequently, while waiting the time set for examination of their stomachs, our freshmen students would, inadvertently or thoughtlessly take a drink from one of the iced water fountains in the building. The stomach thereafter almost invariably showed a large quiescent shadow. It was natural we should be impressed by the relationship between these two occurrences. Further, we learned later that the gastric activity immediately following a drink of cold milk is rapidly diminished. This observation seemed to confirm the suggestion that hypotony and inactivity follow a cold drink of water. We now know that this particular impulse to drink is a symptom of the mental disquiet registered in the gastric behavior. The one is not the cause of the other: both are linked through the mental disquiet.

SUMMARY OF THE WATER PATTERN

The water behavior pattern is quickly summarized. A five-ounce opaque meal increases the amplitude of gastric waves without previously inhibiting them. This increased amplitude is unaccompanied by any evident change in vigor, range, speed or frequency of waves. Amplitude reaches a maximum after two minutes and, after about ten minutes is reduced to the regular neutral depth. The characteristic water waves are accompanied by profuse gastric secretion which is probably responsible for the early disappearance of the water pattern, and by an almost constantly patent pylorus. Hence the gastric shadow remains small and even picks out the rugae in the gastric tube. Accompanying the rapid passage is an evanescent "ballooning" of the gastric tube by temporary downward extension of the Magenblase.

COMPARISON OF WATER AND MILK PATTERNS (FIG. 2)

Earlier in this article we have made a comparison of the dimensions in gastric shadow. In vertical height there is nothing distinctive which would differentiate a water stomach from a milk stomach provided a 5-ounce meal is used in both experiments. We have a very definite feeling that the shadow area of the water stomach is comparatively small, sometimes very small, and that the greatest difference between the milk shadow and the water shadow lies in narrowness of the pyloric vestibule in the water picture. The differ-

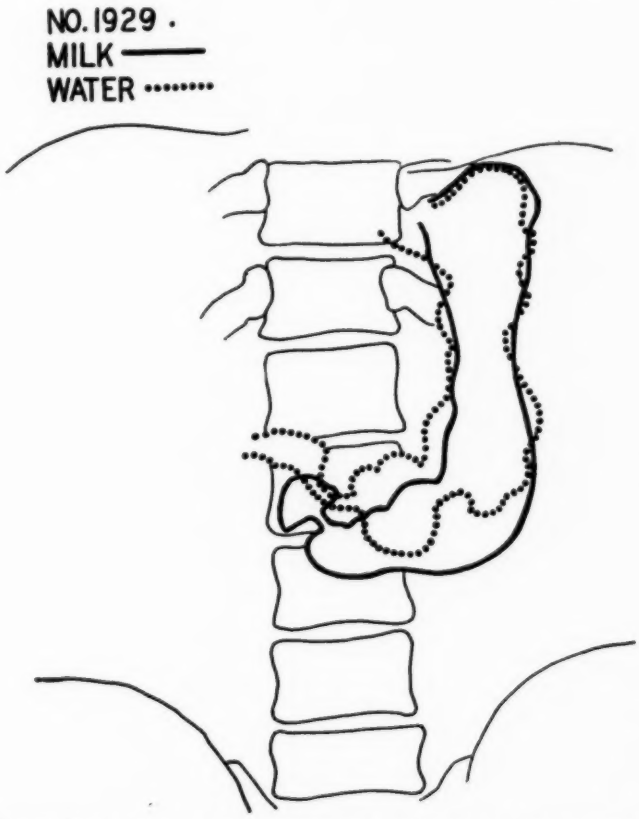

Fig. 2.—Comparison of same stomach after standard milk and water meals. The greater activity, the smaller area, and the slightly shorter length of the stomach shadow after water are apparent. The relative narrowness of the pyloric vestibule is better seen by comparing the water and buttermilk patterns (Figs. 1 and 2).

ence is best expressed figuratively by stating that the water stomach gives the impression of the contents having no weight, whereas, in the milk picture, the weight of the contents sags down the pyloric vestibule. The reader must of course realize that we are speaking figuratively and not stating a fact. It is quite consistent with the impression above recorded that lesser curvature is rarely seen in a milk shadow but is always present in the water shadow. The contained barium exists as a moderate suspension in the milk but as a precipitate in the water. The delineation of lesser curvature in the water shadow and its absence in the milk shadow, therefore, must be due to

difference in the length phase of the circular fibers in pyloric vestibule. Were it not that we desire to avoid the use of tone definitions we might say that the water stomach is more hypertonic than the milk stomach. A corollary of this "hypertony" is the cogwheel outline of greater curvature frequently seen in gastric tube and pyloric vestibule after a water meal.

The differences in motility are equally striking with those of shape of shadow. If the neutral wave pattern has been rendered visible by barium before the milk meal is taken, the neutral waves can be observed to persist for about two minutes at which time the amplitude of the waves begins to diminish so that, about five minutes after the meal, the gastric waves may be very shallow and confined to the pyloric part, or may be replaced by shimmer. It is quite possible that this shimmer is merely a visible expression of the rhythmic arterial pulse in the gastric mucosa which has been described by Carlson.[7] About ten minutes after a five-ounce meal the amplitude of the gastric waves again increases, and within a short time gastric peristalsis is of the usual neutral pattern. The water behavior pattern is practically the converse of this. Under like circumstances the amplitude of gastric waves becomes increased some two minutes after the water meal until about ten minutes after when it begins to diminish to the amplitude characteristic of the neutral pattern. Neither with milk nor water is the rhythm evidently modified.

With the much greater activity of the water stomach, one might imagine that passage through the pylorus would be more apparent than with milk. This is not so: gastric peristalsis causes no churning of stomach contents, nor does it agitate a barium precipitate sufficiently to transform it into a suspension. Therefore while milk passage resembles a puff of smoke of duodenal width, water passage carries definite particles of precipitate in its depths. Passage after a water meal is practically continuous whereas milk passage appears definitely at irregular intervals. The duodenal cap is seen more often after a water meal but is nevertheless quite erratic in its occurrence at roentgenoscopic examination.

COMPARISON OF WATER AND BUTTERMILK PATTERNS (FIGS. 1, 2)

Whereas milk and water merely modify in opposite directions the amplitude of the neutral wave pattern, buttermilk alters the rhythm and frequency as well as the amplitude. The behavior pattern indeed is so changed that it would be forgivable if the observer concluded that the neutral waves had been suspended in favor of a new type. We do not consider this rather common interpretation justified.

The subdivision into "hunger contractions" and digestive waves obscures the fact that each is equally an expression of gastric peristalsis. The gastric shadow after a five-ounce buttermilk meal is relatively large and immediate distention and elongation can usually be observed. At the same time the neutral waves cease and within two minutes the modified pattern begins, waves first appearing at junction of pyloric vestibule and canal, next at mid-pyloric vestibule, then at junction of gastric tube and vestibule. Waves may even start in the mid-tube and be obvious on both curvatures. The shadow is

more even and dense than after milk because the barium suspension is more complete. Passage is represented by a dense massive shadow which is immediately apparent upon swallowing and occurs at regular intervals thereafter. The duodenal cap is well marked or almost complete and may be either intermittent or continuous.

Buttermilk gastric waves are of much greater amplitude than neutral waves, of slower rhythm and much less frequency. It is usual to see only one or two waves at a time after a buttermilk meal whereas the gastric shadow in neutral phase or after a water meal shows three or more wave indentations

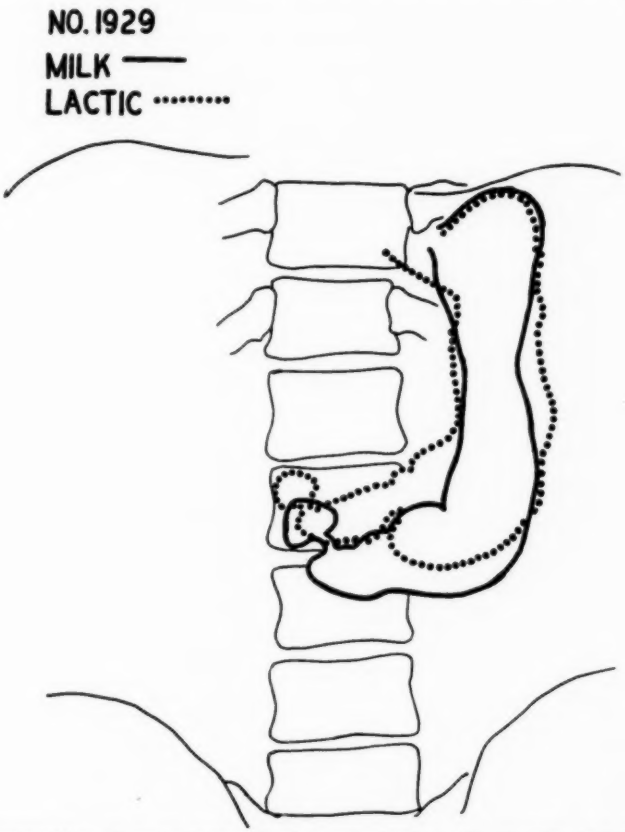

Fig. 3.—Comparison of same stomach after standard meals of milk and lactic acid. In this stomach the lactic shadow is about the same size as that of milk, a little shorter, and somewhat broader. It is also more active and the waves can be seen from immediately below the Magenblase. The relatively broad Magenblase which is so often seen, the shadow intermediate in size between milk and buttermilk, and the elongation are not present in this stomach probably because of its long training.

at once. The buttermilk waves give the impression of forcefulness and vigor, the water waves of activity and speed.

Until the supervention of the neutral phase there is no possibility of confusing water waves with those following a milk meal or a buttermilk meal.

THE LACTIC ACID PATTERN (FIG. 3)

In Table I we have compared the several dimensions of the stomach shadow after a 5-ounce meal of lactic acid (pH_4) with those after milk, water,

and buttermilk. We have already commented upon the undependable character of the statistical average width at the base of the Magenblase. Nevertheless the apparent distinction of stomach width in milk and lactic acid shadows, cited in the table, does indicate a real difference obvious enough upon roentgenoscopic examination. In our comparative records of gastric behavior the relative width of the Magenblase after lactic acid is frequently commented upon. With progressive emptying of the stomach, the characteristic lactic breadth diminishes.

The table indicates a vertical length intermediate between those of water and of milk on the one hand and buttermilk on the other. The oblique elongation undergone by many stomachs detracts somewhat from the value of the specific figures. Some stomachs elongated as much after lactic acid as after buttermilk and a few even more. On the whole the lactic elongation was not so great as that of buttermilk. Curiously enough, although the Magenblase was broader after lactic acid than even after buttermilk, it is the buttermilk stomach which shows the broadest gastric tube. The shadow of the lactic stomach does not give the impression of weight in the pyloric vestibule which is so definite a feature of the buttermilk shadow.

In area the lactic shadow is intermediate between the milk and buttermilk averages. This general impression given by examination of a series of lactic stomachs is that we are looking at water patterns modified quite considerably in the direction of buttermilk behavior.

The relatively broad Magenblase, the gastric tube and pyloric vestibule broader than after water, the less frequent appearance of the cogwheel pattern and of ballooning, distinguish the lactic shadow from that of water. Peristalsis is of moderate amplitude visible from high up in the gastric tube, and distinctly slower than after water. It is also somewhat erratic in its rhythm. The introduction of lactic acid into the stomach, like that of buttermilk, causes temporary inhibition of gastric peristalsis. The neutral waves often appear "frozen" in position as indentations along the greater curvature at or about the following sites: junction of pyloric vestibule and pyloric canal; junction of gastric tube and pyloric vestibule; midgastric tube. Occasionally there is a fourth indentation high up in the gastric tube under the Magenblase. After a few seconds these indentations begin to pulsate and then progressively to initiate waves starting at the most distal site. As the indentations successively originate waves these take on the characteristic behavior pattern of the new meal and the indentations, as such, disappear.

Passage after a lactic meal is distinctly more intermittent than after water and the progress of the gastric contents through the pylorus is consequently slower. The intervals between successive openings of the pylorus are not so long as those after buttermilk. We do not know the relative amount of gastric juice stimulated by water, buttermilk, and lactic acid respectively. We do know, however, that after lactic acid the pylorus is opened far more frequently than after buttermilk though it is not so nearly continuously patent as after water. The relative rapidity of emptying is probably the reason for a gastric shadow smaller than that after buttermilk though larger than a water shadow.

Our observations do not permit us to claim that the gastric juice secreted after a lactic meal flushes the meal from the stomach with the same rapidity as water is flushed away. Our records enable us to state definitely that, twenty minutes after swallowing the meal, gastric peristalsis has once more resumed a neutral character.

COMPARISON OF LACTIC, WATER AND BUTTERMILK PATTERNS (FIGS. 4, 5)

In our previous chapter we have described the several features of shadow form and motility characteristic of lactic acid. It remains only to summarize

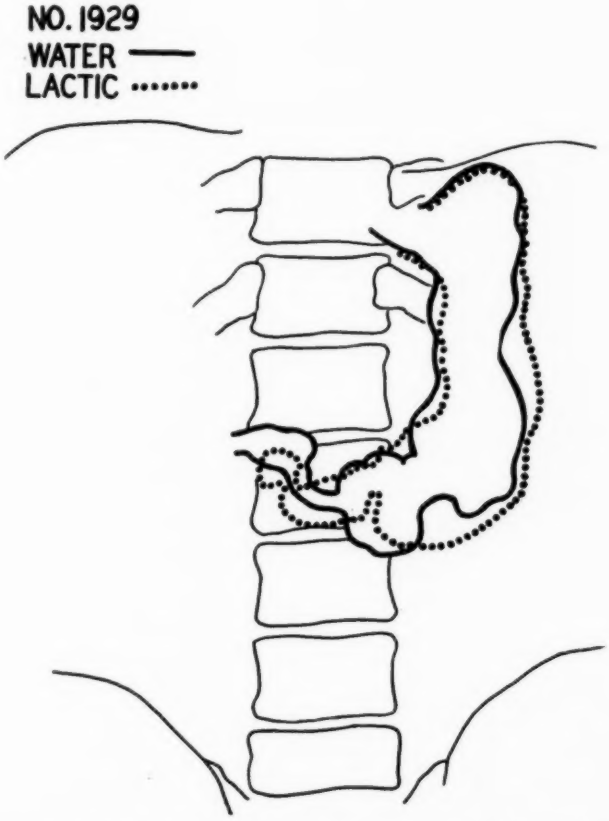

Fig. 4.—The same stomach after standard water and milk meals. Although neither cogwheel nor ballooning features are to be seen in this water stomach, the essential differentiating features are present. The gastric tube is broader, the total area is slightly larger and the activity is different in type and slower in rhythm than after water.

and compare the specific features of lactic, water, and buttermilk patterns.

In contrast with the water pattern, the lactic stomach shadow has a broad Magenblase, a rather broader gastric tube and pyloric vestibule and an elongated outline. The cogwheel and ballooning features so characteristic of water occur more rarely after lactic acid. Peristalsis is definitely changed from neutral type to a slower more erratic rhythm with moderate amplitude of wave. In the development of this change peristalsis ceases temporarily and is replaced by indentations visible along the greater curvature which

first pulsate and then originate the new wave rhythm. Passage is more intermittent and erratic in interval and in amount. Gastric secretion is probably great. After twenty minutes the waves are once more neutral in character.

Comparing the lactic and buttermilk patterns we find that the lactic shadow, broader initially in the Magenblase, is distinctly narrower than the buttermilk shadow in gastric tube and pyloric vestibule and its outline is, on the average, less elongated. The total shadow area is also less.

In the inception of the typical peristaltic rhythm, the lactic and the buttermilk stomach pass through the same phases. Cessation of the neutral

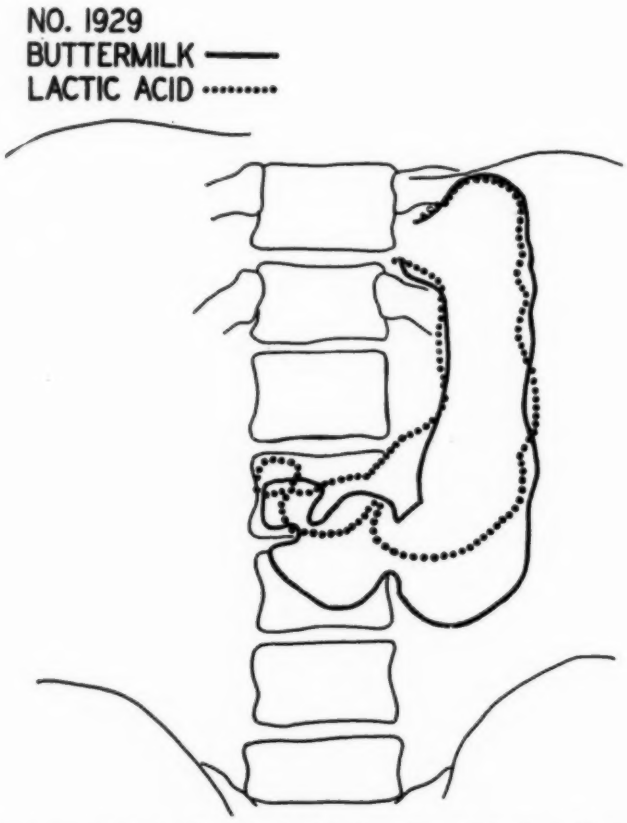

Fig. 5.—The same stomach after standard buttermilk and lactic acid meals. The difference in size of shadow area between lactic and buttermilk is greater than the average in this stomach. The gastric tube is not so narrow as usual after lactic, but the more erratic activity and the smaller shadow area are fairly typical. The importance of these figures lies in their presentation of a single stomach under different stimuli with constant conditions and not in a slavishly exact illustration of average pattern.

rhythm occurs with appearance of indentations on greater curvature which then pulsate and successively originate waves, the first starting at the most distal site. Lactic waves are of about the same amplitude as the buttermilk waves but are more erratic and a little slower in rhythm. They do not give the same impression of forcefulness as buttermilk waves but are easily distinguishable from water waves which are, of course, merely amplified neutral waves.

Lactic passage, like buttermilk, is intermittent but is irregular, whereas

buttermilk passage is regular and rhythmic and occurs in bulky dense shadows usually with a full cap. It may be that the lack of cap delineation and meagerness of passage shadow in lactic acid are due to the fact that barium is a precipitate in a lactic solution as it is in water but is a suspension in buttermilk. The irregularity of lactic acid passage is quite distinctive.

SUMMARY OF THE LACTIC PATTERN

Our experiments with lactic acid convince us that this solution stimulates a gastric behavior bearing the same relation to the water pattern as that which the buttermilk behavior bears to the milk pattern. The modified neutral waves of water are replaced by lactic waves of a slower rhythm just as the modified neutral waves of milk are replaced by the slow rhythm of buttermilk. The almost continuous passage characteristic of water is replaced by an intermittent passage. This is comparable to the increase of the time interval between successive openings of the pylorus which is so obvious in comparison of the buttermilk pattern with that of milk. The gastric shadow of lactic acid bears a relationship to that of water similar to the relationship of the buttermilk shadow to that of milk. The most outstanding feature of the lactic behavior is its erratic character. Area of shadow, peristaltic rhythm and passage are all subject to irregularities in dimension or time-relationship which contrast quite strikingly with the stable behavior pattern of buttermilk. This is a theme which requires further investigation. It serves to emphasize the effect of buttermilk as a gastric stabilizer which brings buttermilk into the same class with sodium bicarbonate and carminatives. This stabilization will be more fully discussed in a later communication.

GENERAL SUMMARY OF WATER AND LACTIC ACID BEHAVIOR PATTERNS

1. This communication describes the results of experiments undertaken to define the influence of water and of lactic acid upon the gastric behavior pattern, in the light of previous studies upon milk and buttermilk.[3]

2. The series here reported upon consists of twenty-eight young male adult healthy stomachs adequately trained and stabilized.[1, 2, 5, 8] It is amplified and confirmed by the study of other series not reported here.

3. The significance of the water picture is better realized after a preliminary investigation of the neutral or "resting" stomach with its neutral waves or "hunger contractions." These neutral waves are of moderate amplitude, of no particular forcefulness, of such frequency that three or four are present at one time, and course over both curvatures of the entire stomach from Magenblase to pylorus.

4. The administration of a 5-ounce water meal (water 4 ounces, barium sulphate 1 ounce) causes no interruption of gastric peristalsis nor any apparent modification in frequency, speed, forcefulness, or range of the waves. The amplitude, however, is deepened in less than two minutes after administration, but returns again to the characteristic neutral phase amplitude after about ten minutes. The pylorus is almost continuously patent after this small water meal and passage is seen as a faint shadow with denser particles in its lower part. This uneven density which characterizes gastric and pass-

age shadows alike is due to the fact that barium is present in the water as a precipitate and not as a suspension.

The small area of gastric shadow, in spite of the known copious gastric secretion, is probably due to the almost continuously patent pylorus. Associated with the small shadow area are the rugous indentations along the greater curvature which result in a cogwheel outline. The Magenblase repeatedly, but momentarily, extends into the gastric tube. This may be a phenomenon accessory to the flushing of contents through the pylorus.

We associate water with an effect opposite to that of milk which reduces the amplitude of gastric waves without altering their neutral frequency, speed or range and which does result in intermittent opening of the pylorus. Its influence is profoundly different from that of buttermilk which interrupts and changes frequency, rhythm and forcefulness as well as amplitude of gastric peristalsis.

5. The relation of the lactic acid pattern to water behavior is similar to the relationship of the buttermilk pattern to that of milk. Commercial buttermilk is simply a lactic milk.

6. Like buttermilk, lactic acid modifies considerably the gastric behavior pattern, replacing the typical features of the neutral phase by a new speed, frequency, forcefulness, and amplitude of waves. The rhythm is much slower than the water rhythm, even slower than that of buttermilk. In the other characters there is no clear distinction between the responses to lactic acid and buttermilk. Range of peristalsis is far more closely linked to volume than to character of contents.

The pylorus is intermittently open and permits passage in large amounts though this is often scarcely visible since the shadow is grey with black particles in its depths as in water passage. The distinctive feature of lactic passage is its erratic character. Doubtless there is marked gastric secretion which flushes out the lactic acid for the wave pattern returns to its neutral character about fifteen minutes after administration of the small standard 5-ounce meal. It is probably due to the frequent and recurrent openings of pylorus that the gastric shadow is intermediate in size and length between those of water and buttermilk. The rugous indentations and "ballooning" sometimes seen, but less frequently than after water, are probably brought about in similar manner.

The stable buttermilk pattern contrasts with the erratic lactic acid behavior although the latter is, as it were, an imperfect caricature of the former.

ABSTRACT

1. A series of twenty-eight young, healthy, stabilized and trained adult male stomachs is used as a type series for the study of gastric behavior after administration of a 5-ounce water or lactic acid meal.

2. Water is found to modify amplitude but not range, frequency, speed or forcefulness of gastric waves which, except in amplitude, remain of neutral type.

The water modification has commenced within two minutes and is lost again in less than fifteen. Patency of pylorus neutralizes large gastric secre-

tion, keeps size of shadow small, induces "cogwheel" rugous indentations on greater curvature and sucks gas from Magenblase into gastric tube (ballooning).

3. Water and milk modify the neutral pattern in opposite manner.

4. The relation of the lactic pattern to that of water is, in general, the relation of the buttermilk pattern to that of milk. The gastric waves are reduced in speed and frequency and increased in amplitude and force. There is a definite break with the neutral pattern and a slight pause before the substitution of the lactic response. The pylorus is less frequently open, the gastric shadow is greater, "cogwheel" and "ballooning" less marked and the entire behavior pattern is erratic in character.

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LABORATORY METHODS

PHOSPHORUS METABOLISM*†

I. A System of Blood Phosphorus Analysis

By Guy E. Youngburg, Ph.G., Ph.D., and Mamie V. Youngburg, A.B.,
Buffalo, N. Y.

THE following is a colorimetric system, utilizing the principle of Osmond, who in 1887[1] obtained a blue color by reducing phosphomolybdate with stannous chloride. In 1921 Deniges[2] utilized this sensitive reaction as the basis for the quantitative determination of phosphorus and of arsenic. In 1927 Kuttner and Cohen[3] developed the method further by studying the most favorable concentrations of the reagents used and applying the method primarily to the determination of inorganic phosphate and of calcium in pus, but also to plasma and spinal fluid. In their paper Kuttner and Cohen give no figures for phosphorus either for pus or spinal fluid by their method and so far as we can find no subsequent paper has been published by them on this subject.

Truog and Meyer[4] in 1929 published a paper on "Improvements in the Deniges Colorimetric Method for Phosphorus and Calcium." They were not aware of the paper of Kuttner and Cohen. Truog and Meyer studied the optimum concentration of reagents for the development of the blue color except that they apparently paid little attention to the concentration of stannous chloride and concluded to use 6 drops per 100 c.c. They particularly studied the preparation and keeping qualities of the stannous chloride as well as the effect of interfering substances. Their method is designed for phosphates in water, soil, minerals, fertilizers, etc. They find optimum concentrations of the reagents for color development which are far different from those of Kuttner and Cohen. We have checked up both concentrations and a discussion is found later in this paper.

During the last twenty years a number of similar colorimetric phosphorus methods have appeared, using other reducing agents than stannous chloride. The subject is discussed by Kuttner and Cohen and by Truog and Meyer in the papers mentioned and will not be repeated here, but we want to state our own results in comparing the various methods.

We find the Kuttner and Cohen method, as adapted and extended by us, to be definitely superior to the Bell and Doisy, Benedict and Theis, and also the Fiske and Subbarow[5] method, which last has largely superseded the various other methods.

*From the Department of Biological Chemistry, University of Buffalo Medical School. Received for publication, April 21, 1930.

†Since this was written the paper of Kuttner and Lichtenstein has been published, J. Biol. Chem. 86: 671, 1930.

The superiority of the stannous chloride reduction lies in the facts that the color produced is, as Kuttner and Cohen state, 12 to 15 times more than that of Fiske and Subbarow; the reagents used are entirely inorganic compounds which deteriorate inappreciably except it be the dilute stannous chloride solution, but it is an insignificant task to make it by simple dilution from the permanent stock solution once a week.

Objections to the stannous chloride reduction of phosphomolybdate have been made because the concentration of the reagents in the final solution must not vary much. This criticism is not valid for the molybdate and stannous chloride because they are simply added in definite quantity to the final color solution and with ordinary care in pipetting they come well within the necessary limits. The acidity, using sulphuric acid, however, requires some attention. The limits for maximum color development is, as indicated by Kuttner and Cohen, 0.9 to 1.05 N sulphuric acid. This is not difficult to obtain when the acid is added directly to the final color solution, as for example in the determination of inorganic phosphate in plasma or serum. But in the determination of total phosphorus, lipid phosphorus, etc., in blood, where oxidation of organic matter must be carried out by digestion in sulphuric acid with heat, appreciable acid may be volatilized unless care is taken.

We have overcome any significant acid loss, however, by carrying out the heating in 150 × 18 mm. Pyrex test tubes, placed in a slanting position on an 8-inch diameter electric hot plate. By arranging wooden supports on two sides, 10 or 12 tubes can conveniently be digested at one time. The tops of the supports have nail pegs at intervals for holding the tops of the tubes. The heat must not be too great, although with an ordinary 110-volt 3-heat plate the full heat is satisfactory. The acid loss comes within the limit shown necessary by Kuttner and Cohen, viz.: that if 1 N sulphuric acid is taken it must not be reduced below 0.95 N. This, of course, can be shown by titration of the mixture after digestion.

CHOICE OF OXIDIZING AGENT IN PHOSPHORUS DETERMINATIONS

It is difficult to remove all of the nitric acid from any sulphuric acid digestion mixture and its presence in minute amount either inhibits the blue color production or gives a yellow tint to the blue, depending on the concentration. It was necessary to abandon the nitric acid entirely in this method. Martland and Robison[6] have also abandoned it but their reason was on account of the etching of the glass by phosphoric acid when heating with sulphuric acid at the high temperature required for oxidation with nitric acid. Perhydrol was substituted and has proved here, as in many other oxidation procedures, to be very satisfactory. It was first used by Baumann[7] in phosphorus determinations. When added as described below it causes most rapid oxidation and any excess present is completely decomposed or volatilized. In any blood phosphorus determination indicated below not more than 7 drops of perhydrol is needed. Since using it we have always obtained a pure blue for color comparison. Perhydrol deteriorates slowly, but that is insignificant if the stock is kept in the refrigerator.

OPTIMUM CONCENTRATION OF REAGENTS FOR MAXIMUM COLOR PRODUCTION

Kuttner and Cohen appear to be the first to study the optimum conditions for stannous chloride reduction of phosphomolybdate. This involves the concentrations of sulphuric acid, molybdate and stannous chloride. While other investigators have studied the acid and molybdate concentrations in connection with the use of other reducing agents than stannous chloride, they seem to have omitted the use of this admirable reducing agent either because they were not aware of it for biologic work or they thought that its effect on the color production was too great and the concentration limits too narrow to permit its use. Fiske and Subbarow in their otherwise very thorough paper do not mention the use of stannous chloride. Since we are concerned here only with the optimum conditions for stannous chloride reduction we will not treat of the acidity and molybdate concentrations used by Bell and Doisy, Briggs, Fiske and Subbarow, etc., in their methods, except to mention that by use of different reducing agents they use widely different concentrations of acid and molybdate.

The following are the concentrations used by Kuttner and Cohen and by Truog and Meyer in their final color solutions:

	SULPHURIC ACID	MOLYBDATE	STANNOUS CHLORIDE
	NORMALITY	PER CENT	PER CENT
Kuttner and Cohen	1.0	0.75 (Na salt)	0.02
Truog and Meyer	0.4	0.10 (NH_4 salt)	0.0075

The reason for the widely different concentrations used is perhaps the fact that Truog and Meyer did not employ a lower concentration of molybdate than 2 c.c. of 5 per cent ammonium molybdate per 100 c.c. They did try a higher concentration but obtained less color. Also Truog and Meyer do not state that they used a sufficiently wide range of stannous chloride concentration. They appear not to have done so. It must be further observed that Truog and Meyer used ammonium molybdate, while Kuttner and Cohen used the sodium salt. With the purest samples of these salts which we were able to procure and which were practically phosphorus-free, and working under the method otherwise prescribed by Truog and Meyer, we obtained approximately 30 per cent more color with the ammonium salt (4 c.c. of 2.5 per cent molybdate solutions were used in each case). By determining the color production on a standard solution of phosphate using the Kuttner and Cohen method adapted by us, and the Truog and Meyer method, we obtained a reading of the colorimeter of 22.5 mm. for the Truog and Meyer color solution, when using the Kuttner and Cohen solution as the standard and set at 20 mm. The Kuttner and Cohen color development was therefore 12.5 per cent greater.

We have been able to verify Kuttner and Cohen in acid, molybdate and stannous chloride concentrations for optimum color production and have therefore used these concentrations in the work reported here.

PROPORTION OF COLOR PRODUCED TO PHOSPHATE PRESENT

This factor is an important one in all of the methods depending on the comparison of the blue color from phosphomolybdate reduction by various reagents. Martland and Robison[6] state that they find "the intensity of the color is proportional to the amount of phosphate within very wide limits." But Stanford and Wheatley[8] find that the color is not strictly proportional and cite the deviations found. They conclude that the unknown and standard should not differ by more than 30 per cent if the results are to be free from uncertainty.

Our experiments tend to confirm those of Stanford and Wheatley.

When we used 0.02 mg. phosphorus in the standard, the amounts of phosphorus indicated for the other tubes, and comparing in a Spencer Dubosque colorimeter, we obtained the following results:

P. TAKEN	P. FOUND	P. RECOVERED
mg.	mg.	per cent
0.010	0.0110	110.0
0.015	0.0150	100.0
0.020	(standard)	
0.025	0.0240	96.0
0.030	0.0276	92.0
0.040	0.0354	88.5

The results show that if the color intensity is greater than the standard the results will tend to be low, while if less, they will tend to be high. There is then, a serious error unless the unknown comes within about 30 per cent of the amount of the phosphate in the standard. The chloroform used as a preservative in the standard phosphate solution is without any effect on the color.

ADAPTATION OF THE METHOD TO BLOOD PHOSPHORUS DETERMINATIONS

We desired to make determinations of the usual classes of phosphorus compounds in plasma, corpuscles and whole blood by some system of analysis which would not require more blood than could ordinarily be drawn without objection and which would permit the use of an ordinary accurate colorimeter. Specifically the following determinations were to be made:

In whole blood: total phosphorus.

In plasma and corpuscles: total, inorganic phosphate, total acid-soluble, and lipid phosphorus.

For this purpose 10 c.c. of the final color solution in each determination is sufficient because it fills the colorimeter cups about two-thirds full.

For the standard phosphate solution 0.02 mg. of phosphorus in 10 c.c. is about the most favorable for color comparisons. When the standard is set at 20 mm., it permits reading unknowns varying approximately 30 per cent above or below 0.02 mg. with a good degree of accuracy.

By calculating from the above data and by considering other data on blood phosphorus, 20 to 25 c.c. of blood should be needed, but at the same time this amount would be ample for redeterminations when desired.

In our determinations requiring oxidation of organic matter over the hot plate we add half of the sulphuric acid as a part of the digestion mixture and half to the sodium molybdate solution. This gives ample volume to the digesting mixture so that there is no danger of solidification of the contents nor loss of acid through overheating of too small a quantity. This principle has already been utilized by Martland and Robison and Fiske and Subbarow.

In the determination of the inorganic phosphate, where digestion is not required, we add all of the acid to the sodium molybdate. We thus need two molybdate-acid solutions, both equal in concentration of sodium molybdate but one containing twice as much sulphuric acid as the other.

The use of digestion tubes smaller than the 200×25 mm. customarily used for micro digestion is of considerable advantage; 150×18 mm. Pyrex test tubes graduated at 10 c.c. require only 0.5 c.c. of the 10 N sulphuric acid for any of our determinations. And we make no transfer of the contents of the tubes until pouring into the colorimeter cups. For convenience similar tubes are used for the determinations where no digestion is required. It is obvious that the tubes must be very accurately graduated.

In reference to the concentration of trichloracetic acid in the precipitation of the blood proteins we insure complete precipitation according to Fiske and Subbarow by having a concentration of 8 per cent of the acid present. During the determination of the inorganic phosphate the filtrate containing the 8 per cent of trichloracetic acid is incidentally diluted 5 times, or to 1.6 per cent concentration in the final color solution. This determination is the only one in which the trichloracetic acid is not removed prior to color development.

In adjusting the acidity for maximum color production we at first thought that the trichloracetic acid present in the solution for color development would have an effect equal to that of sulphuric acid since the affinity constants of these acids is very nearly the same. But this is not the case; the organic acid can vary as much as 4 per cent before it begins to interfere with maximum color production. Kuttner and Cohen have already found this and our experiments confirm theirs.

For the lipid phosphorus determination the well-known alcohol-ether extraction of Bloor is used. The filtrates are evaporated on the hot plate before adding acid digestion mixture. The residue is easily oxidized with perhydrol.

SPECIAL REAGENTS REQUIRED

1. Ten N Sulphuric Acid.—450 c.c. of concentrated sulphuric acid, c.p. are added to 1200 c.c. of water. This solution is titrated and diluted to make 10 N.

2. Ten per cent Trichloracetic Acid, c.p.—Inferior acid must be purified by distillation before diluting to 10 per cent.

3. Alcohol-ether Mixture.—Bloor's mixture of 3 volumes redistilled 95 per cent alcohol and 1 volume of redistilled ether.

4. Molybdate-sulphuric Acid Mixture.—Solutions A and B are made as follows:

A. Mix 50 c.c. of 7.5 per cent sodium molybdate, c.p. (P-free) and 50 c.c. of 10 N sulphuric acid.

B. Mix 50 c.c. of 7.5 per cent sodium molybdate, c.p. (P-free), 25 c.c. of water, and 25 c.c. of 10 N sulphuric acid.

We used Kaulbaum's sodium molybdate at first but were later unable to purchase this brand. We had difficulty with other samples in that they contained phosphorus. However, after making known our needs to the manufacturer we obtained a satisfactory chemical.

5. Stannous Chloride Solutions. (Stock and dilute solutions as prescribed by Kuttner and Cohen.)

Dissolve 10 gm. of Kaulbaum's stannous chloride in 25 c.c. of concentrated hydrochloric acid, c.p. Store in a brown glass-stoppered bottle.

Dilute 1 c.c. of the above stock solution to 200 c.c. with water. This diluted reagent may be used with safety for a week, but should then be discarded and a new dilution made.

6. Standard Phosphate Solution.—Dissolve 0.4394 gm. pure, dry monopotassium phosphate (KH_2PO_4) in water to make 100 c.c. One c.c. = 1 mg. P.

Dilute 1 c.c. of the above stock solution to 100 c.c. One c.c. = 0.01 mg. P.

Preserve both solutions with chloroform.

7. Perhydrol.—Superoxol Merck (Blue Label, 30 per cent H_2O_2, 0.0005 per cent P_2O_5). Purchased in 50 gm. containers and kept in the refrigerator when not in use.

Procedure.—One c.c. of oxalated blood is first diluted to 20 c.c. with physiologic saline solution and set aside for the determination of total phosphorus.

The remaining blood is centrifuged for one-half hour at about 2200 r.p.m. For our purpose we used 20 to 25 c.c. of blood and centrifuged in graduated tubes, observing the volume of cells and plasma. Most of the plasma is then drawn off carefully with a fine-tipped pipette improvised from 8 mm. glass tubing, a rubber tube connection being attached for gentle suction from the mouth of the operator. The plasma still remaining above the corpuscles is likewise removed, with a small portion of the cells, and is discarded. In this way there is as good a degree of separation as is possible.

The trichloracetic acid filtrates, alcohol-ether extracts, and the dilutions for total phosphorus in corpuscles and plasma are then made for all of the determinations given below. It is to be noted that the same filtrate is used for acid-soluble- and total acid-soluble-phosphorus determinations.

Determination of acid soluble phosphorus (inorganic phosphate) in plasma or serum.—Four c.c. of 10 per cent trichloracetic acid are transferred to a 25 c.c. Erlenmeyer flask or an ordinary test tube, 1 c.c. of plasma or serum is added while mixing and, after shaking a few times, the mixture is filtered through an ashless or phosphorus-free filter paper. Two c.c. of the filtrate are transferred to a Pyrex test tube graduated at 10 c.c., and 2 c.c. of the standard phosphate solution (1 c.c. = 0.02 mg. P) are transferred to a similar tube. To each tube is then added about 6 c.c. of water, 2 c.c. of molybdate-sulphuric acid solution A, and finally 1 c.c. dilute stannous chloride solution and water to the 10 c.c. mark. (The addition of the stannous chloride, water to the mark, and mixing

should be done without delay. It is well to deliver the stannous chloride from the pipette under some pressure from the mouth.)

Compare in the colorimeter after one minute, ordinarily setting the standard at 20 mm.

Calculation.—When 2 c.c. of the filtrate is taken and the standard is set at 20 mm.: 20/reading = mg. P per 100 c.c. plasma or serum.

Determination of total acid-soluble phosphorus in plasma or serum.—A filtrate as for acid-soluble phosphorus is prepared, viz.: by slowly mixing 1 c.c. of plasma or serum with 4 c.c. of 10 per cent trichloracetic acid and filtering; or if both determinations are to be made, enough filtrate is prepared in one process by taking a multiple of the above. We usually take 8 c.c. of the trichloracetic acid and 2 c.c. of plasma.

Two c.c. of the filtrate are then digested with 0.5 c.c. of 10 N sulphuric acid and a silica pebble (from broken silica-ware) in a Pyrex test tube graduated at 10 c.c., it being placed in a slanting position over the electric hot plate, the bottom of the tube resting on the hot plate and the top of the wood support placed beside and about one and a half inches above the latter.

When the trichloracetic acid is all evaporated and the mixture turns brown, the tube is temporarily removed and after cooling for about thirty seconds a drop of perhydrol is allowed to fall into the mixture. The tube is replaced and the heating continued until oxidation is complete, more additions of perhydrol being made if necessary.

The tube is then placed in a rack, allowed to cool somewhat, about 2 c.c. of water are added and the contents are heated to boiling to insure conversion of any meta- or pyro-phosphate that may have been formed, into orthophosphate. The silica pebble is removed. About 4 c.c. of water and 2 c.c. of molybdate-sulphuric acid solution B are then added.

The standard is then prepared by transferring 2 c.c. of the standard phosphate solution (1 c.c. = 0.02 mg. P) to a similar tube, and adding about 4 c.c. of water and 2 c.c. of molybdate-sulphuric acid solution A.

To each tube is then added without delay 1 c.c. of dilute stannous chloride solution and water to the 10 c.c. mark and the contents are quickly mixed.

Compare in the colorimeter after one minute, ordinarily setting the standard at 20 mm.

Calculation.—When 2 c.c. of the filtrate are taken and the standard is set at 20 mm.: 20/reading = mg. P per 100 c.c. of plasma or serum.

Determination of lipid phosphorus in plasma or serum.—Sixteen to 18 c.c. of alcohol-ether mixture are transferred to a wide test tube (best 150 × 20 mm.) graduated at 20 c.c., and 1 c.c. of plasma or serum are dropped in. After mixing, the tube is placed in a boiling water-bath and the contents are heated to boiling. The tube is removed from the water, allowed to cool to room temperature and the contents are made up to the 20 c.c. mark with more alcohol-ether mixture. After mixing, the contents are filtered.

Four c.c. of the filtrate are transferred to a 150 × 18 mm. Pyrex test tube graduated at 10 c.c., a silica pebble is added, the tube is placed in a wire rack over the hot plate and the contents are evaporated to dryness. The wire rack must have a wire-net bottom.

Five-tenths c.c. of 10 N sulphuric acid is transferred to the tube and digestion is carried out over the hot plate as indicated for total acid-soluble phosphorus, adding the necessary amount of perhydrol.

The remaining technic is the same as for total acid-soluble phosphorus in plasma or serum.

Calculation.—When 4 c.c. of alcohol-ether filtrate is taken and the standard is set at 20 mm.: 20/reading × 10 = mg. lipid P per 100 c.c. of plasma or serum.

Determination of total phosphorus in plasma or serum.—One c.c. of plasma or serum is transferred to a test tube graduated at 20 c.c., 0.2 per cent sodium chloride solution is added to make the contents 20 c.c. and mixed.

Two c.c. of the diluted plasma or serum are taken, the procedure being the same as for total acid-soluble phosphorus. But we usually add the 10 N sulphuric acid before evaporating the water. Foaming may be obviated by directing an air current into the tube. For this purpose a small diameter rubber tube is tipped at one end with a glass tube rather finely drawn out, the other being connected with the compressed air tap.

Calculation.—When 2 c.c. of the diluted plasma or serum are taken and the standard is set at 20 mm.: 20/reading = mg. P per 100 c.c. of undiluted plasma or serum.

Determination of acid-soluble phosphorus (inorganic phosphate) in corpuscles.—The procedure is the same as for acid-soluble phosphorus in plasma or serum.

Determination of total acid-soluble phosphorus in corpuscles.—The procedure is the same as for total acid-soluble phosphorus in plasma or serum, except that the filtrate first prepared is diluted 1 to 4, and 1 c.c. of this diluted filtrate is taken.

Calculation.—When 1 c.c. of the diluted filtrate is taken and the standard is set at 20 mm.: 20/reading = mg. P per 100 c.c. of corpuscles.

Determination of lipid phosphorus in the corpuscles.—The procedure is the same as for lipid phosphorus in plasma or serum.

Determination of total phosphorus in corpuscles.—The procedure is the same as for total phosphorus in plasma or serum, except that the corpuscles are diluted 1 to 40 with 0.2 per cent sodium chloride and only 1 c.c. is used.

Calculation.—When 1 c.c. of the 1 to 40 dilution of corpuscles is taken and the standard is set at 20 mm.: 20/reading = mg. total P per 100 c.c. of corpuscles.

Determination of total phosphorus in whole blood.—The procedure is the same as for total phosphorus in plasma or serum, except that only 1 c.c. of the 1 to 20 dilution is used.

Calculation.—When 1 c.c. of the 1 to 20 dilution is taken and the standard is set at 20 mm.: 20/reading = mg. total P per 100 c.c. of whole blood.

SUMMARY AND CONCLUSIONS

The colorimetric method whereby phosphomolybdate acid is reduced by stannous chloride, is adapted to the various blood phosphorus determinations.

Stannous chloride as the reducing agent is distinctly superior to other reducing agents that are in vogue.

The method for inorganic phosphate in plasma or serum should prove to be very useful in routine determinations in hospitals and elsewhere.

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HEATED BLOOD AND BLOOD DERIVATIVES FOR CULTIVATION OF THE HEMOGLOBINOPHILIC ORGANISMS*

By Robb Spalding Spray, Ph.D., Morgantown, W. Va.

IN 1927 I[1] described a blood-clot digest medium for the cultivation of hemoglobinophilic and other organisms. This medium apparently met favor at the hands of others who have used it, but its preparation is somewhat involved and time-consuming. Further studies have developed a simplified procedure resulting in several forms of blood derivatives which have the advantage of ease of preparation, and which appear to give even superior results.

In making the so-called "chocolate agar" sterile blood is the prime essential. Facilities, time, and animals are frequently not available in the small laboratory. Since, in its preparation, the blood is heated from 95 to 96° C., it would appear that it might as well be autoclaved to insure sterility.

If the blood is first added to agar, and the mixture be autoclaved, the serum coagulates and separates from the agar. If whole blood is autoclaved, it, of course, coagulates. Thus, neither procedure is possible. If blood is first defibrinated by shaking with glass beads, the corpuscles centrifuged and washed thrice with saline, then made up to blood volume with saline, it will still coagulate if autoclaved. But, if we add to one volume of the saline-corpuscle suspension 3 volumes more of saline, we can autoclave, and the

*Professor of Bacteriology, Medical School, The West Virginia University.
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result is a soft semicoagulum which will settle out on standing like fresh defibrinated blood. Upon slight shaking, however, a diffuse suspension again results. This suspension is sterile, and we may call it Preparation No. 1.

If we start again with the washed corpuscles and, after the third washing, instead of adding saline we add distilled water to make to the original blood volume, the corpuscles hemolyze, and the suspension will also coagulate on autoclaving. If, to one volume of this hemolyzed suspension we add 3 volumes more of distilled water and autoclave, again a soft coagulum forms, which is softer than that formed in saline, and which gives even a better, smoother creamy suspension. This may be called Preparation No. 2.

Either Preparation No. 1 or No. 2 may be added to sterile agar by the following precedure: Prepare preferably a "vitamine" agar, such as described by me,[2] which is made up to 1.8 per cent agar (to compensate for the blood fluid added), and autoclave in 100 mil., or multiple units, in flasks. Sterilize 20 mil. volumes of blood preparation No. 1 or No. 2 in small flasks. Cool the agar and blood fluid to about 45° C. and add the blood to the agar with the usual aseptic precautions of flaming lips of flasks. Rotate to mix and pour plates. Cool the plates quickly, and a smooth, opaque medium results. When first poured the color is a pale brown, which turns to reddish brown on standing a few minutes.

On this medium H. influenzae grows well. The Pneumococcus produces a broad zone of decolorization around the colonies, while most strains of Streptococcus viridans show only a moderate greenish ring. Streptococcus hemolyticus is inactive.

We have used these two blood preparations with success for the past four years in carrying stock cultures of many strains of Influenza, Pneumococcus and Streptococcus.

Realizing that it might be advantageous to incorporate the plasma as well as the corpuscles, we sought a method which might be applied to the whole blood to give similar results. If we add to one volume of blood in order, 1, 2, 3, and 4 volumes of distilled water and then autoclave, the first two, and sometimes three, will coagulate too firmly. With 4 volumes, however, and often 3½ volumes of water, a soft fluid suspension results.

We have, therefore, recently used this latter method, which we shall call Preparation No. 3, and feel that from our comparative tests it is superior to the others. In preparing this latter, we take one volume of fresh, mechanically defibrinated blood and add 4 volumes of distilled water; mix this well and distribute in various amounts in flasks and tubes. Autoclave, and when cool, paraffin the plugs. Upon removal from the autoclave the solution is a pale brown, but upon cooling the color returns to a rich chocolate reddish-brown. Of this Preparation No. 3 we add, by the above method, 25 mil. to 100 mil. of sterile agar (1.9 per cent agar), and pour plates.

Using the usual methods of aseptic tubing, we prepare at the same time a lot of slants in tubes. These slants are chilled quickly, and are always incubated for sterility before inoculating. While we would not advocate the procedure, we have found our old stock cultures of H. influenzae survive on these slants, sealed with paraffin, as long as one month.

These preparations, then, offer several advantages over the routine procedure of making "chocolate agar." The blood is obtained at the slaughter house with no necessary aseptic precautions. Any desired volume may be obtained. No intricate technic is involved, and the diluted preparations, when autoclaved, are of known sterility, and keep apparently indefinitely if protected against evaporation by sealing the containers. They may be kept on ice or at room temperature, and finally, they are at hand at all times to make one or one hundred freshly prepared plates or tubes.

During the course of this study we have worked closely with the Digestive Ferments Company,* with the result that two dehydrated preparations have been made available for experimental work. The first is termed "Bacto-Hemoglobin," and is equivalent to our Preparation No. 2, being made by dehydration of the washed corpuscles. The second is termed "Bacto Whole Blood," and is made by diluting and then dehydrating the whole blood, resulting in the equivalent of our Preparation No. 3.

Each of these is used by dissolving completely 2 gm. of the powder in 100 mil. of distilled water at about 50° C. A single layer of gauze is spread over a funnel and the solution passed rapidly through to strain out any slight insoluble precipitates. The usual filtration through cotton is to be avoided, as it retains too much of the corpuscular substance. The solution is then autoclaved.

At the same time 100 mil. units of *double-strength agar* are prepared, using preferably "vitamine" agar.† The agar is autoclaved separately and, after cooling to 45° C., the blood solution is added with aseptic precautions to the agar, rotated to mix, and poured in plates or tubed as desired.

Recent comparative studies show the dehydrated forms to give results comparing equally favorably with those of the fresh blood dilution. The use of the "Whole Blood" seems to confer more growth-accessory properties than the "Hemoglobin," since it contains the plasma as well as the corpuscles, although the latter gives a better and richer blood color to the medium.

These dehydrated forms would seem almost ideal for the small laboratory, since they may be kept in stock available at any time, and obviate the necessity of collecting, washing corpuscles, or diluting the blood. Sample lots have been tested in a number of laboratories, other than our own, and very favorable results have been reported.

We might add that, aside from their use in culture media, these preparations offer a convenient form of blood for studies of the chemical constituents, especially those of the hemoglobin fraction.

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*Bacteriological Laboratories, Digestive Ferments Company, Detroit.

†"Bacto-North Gelatin Agar," prepared by the Digestive Ferments Co., according to my formula, is very satisfactory and convenient for the purpose.

THE EFFICIENCY OF BLOOD CULTURES*

With Report of a New Method Based on Complement Fixation

By Roy R. Kracke, M.D., and Harry E. Teasley, M.D., Emory University, Ga.

THE importance of blood cultures has long been recognized, chiefly as a diagnostic aid in practically all types of infectious diseases. This importance becomes more manifest when it is considered that in some conditions specific therapy may be instituted only after a positive diagnosis by means of a blood culture. This can be illustrated by the successful type I pneumococcus serum therapy in a bacteremia due to that organism. Also diagnoses are often dependent on the outcome of a blood culture, as in typhoid fever, the various streptococcic and staphylococcic septicemias, and other infectious conditions. Positive blood cultures in these conditions always mean that the clinician is better able to evaluate the condition of the patient, can institute more appropriate systemic and symptomatic treatment, and has more accurate data on which to base his prognosis.

The efficiency of all laboratory procedures can be expressed only in a relative way. For example: a positive malarial smear or the finding of tubercle bacilli in sputum are examples of 100 per cent efficiency. The efficiency of the negative findings in these types of examinations is not so significant. A positive blood culture is also an example of a relatively efficient laboratory procedure, only when positive. Even then because of the possibility of contaminating organisms, this efficiency is materially reduced. A negative blood culture obtained with the methods in current use is of little significance, for reasons that we shall attempt to bring out in this paper.

A general review of the literature shows that blood cultures as employed today with the current methods are relatively inefficient as only about an average of 10 to 15 per cent positive cultures are obtained. Such a laboratory procedure with positive findings so low in cases that clinically warrant such an examination certainly could be justly considered a relatively inefficient procedure.

Burbank[1] working with arthritis states that routine blood cultures gave very few positive results, and Straus[2] states that 10 per cent positives in typhoid cultures in his series would be a liberal estimate. Lawson[3] obtained only 10.5 per cent positives in 2092 cultures at Bellevue hospital over a period of two years. Our own records at Wesley Memorial hospital show that in using the older methods in the last three years, we obtained similar results.

*From the Department of Bacteriology, School of Medicine, Emory University.
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Many methods for taking blood cultures are in current use, and investigation shows that almost every hospital has a method of its own. This may explain some of the reports of high percentages of cultures in some institutions. For instance Fox and Leaman[4] report 25 per cent positives using the old methods. It is certainly evident that there is a considerable variation in blood culture technic and a corresponding variation in the percentage of reported positives.

Many various types of culture media have been employed and many different technical procedures advocated with wide variations in the technic. For example, Todd and Sanford[5] recommend several methods, using from 5 to 20 c.c. of blood and planting this on various fluid and solid culture media. Stitt[6] suggests a similar method by collecting the blood through a suction apparatus and into sodium citrate solution and distributing it over various types of culture media. There is no uniformity of methods, but one outstanding fact is evident, and that is that the quantity of blood used is small and the quantity of medium is correspondingly large.

The following experiments illustrate why dilutions are necessary. The following experiment was carried out to determine the optimum dilution of blood in culture media for blood culture.

Rabbit No. 1.—Inoculated intravenously with 1 c.c. of a saline suspension of an 18 hour culture of freshly isolated Staphylococcus aureus grown on plain agar. One c.c. of this suspension contained approximately 10,000 organisms. Fifteen minutes later 20 c.c. of blood was withdrawn from the animal's heart, and immediately planted out on the following culture media:

TABLE I

AMOUNT OF BLOOD	CULTURE MEDIUM	GROWTH
1 c.c.	none	none
1 c.c.	1 c.c. bouillon	none
1 c.c.	2 c.c. bouillon	none
1 c.c.	5 c.c. bouillon	none
1 c.c.	10 c.c. bouillon	1-plus
1 c.c.	20 c.c. bouillon	2-plus
1 c.c.	50 c.c. bouillon	1-plus
1 c.c.	75 c.c. bouillon	1-plus
1 c.c.	100 c.c. bouillon	1-plus

Rabbit No. 2.—Inoculated with the same organism at the same time, but using a larger number of bacteria, estimated at 100,000 per c.c. The following results were obtained:

TABLE II

AMOUNT OF BLOOD	CULTURE MEDIUM	GROWTH
1 c.c.	none	none
1 c.c.	1 c.c. bouillon	none
1 c.c.	2 c.c. bouillon	none
1 c.c.	5 c.c. bouillon	1-plus
1 c.c.	10 c.c. bouillon	2-plus
1 c.c.	20 c.c. bouillon	4-plus
1 c.c.	50 c.c. bouillon	3-plus
1 c.c.	75 c.c. bouillon	2-plus
1 c.c.	100 c.c. bouillon	2-plus

The above experiments clearly indicate that the optimum dilution for blood and media ranges around 1-20. The most luxuriant growths were obtained at that dilution in both experiments. In higher dilutions the growth is probably as luxuriant, but the larger quantities do not seem to be necessary. This dilution is in conformity with those employed by the majority of laboratory workers and bears out the old precaution to use small quantities of blood in large quantities of media.

In order to further confirm the findings noted in the tables given above we carried out a similar experiment as follows:

Rabbit No. 3.—Inoculated with a one c.c. suspension of freshly isolated Staphylococcus aureus, containing approximately 10,000 organisms per c.c. Fifteen minutes later 25 c.c. of blood was withdrawn from the heart and planted out on the following culture media:

TABLE III

AMOUNT OF BLOOD	CULTURE MEDIUM	GROWTH
1 c.c.	none	none
1 c.c.	1 c.c. bouillon	none
1 c.c.	2 c.c. bouillon	none
1 c.c.	3 c.c. bouillon	none
1 c.c.	4 c.c. bouillon	none
1 c.c.	5 c.c. bouillon	none
1 c.c.	6 c.c. bouillon	none
1 c.c.	7 c.c. bouillon	none
1 c.c.	8 c.c. bouillon	none
1 c.c.	9 c.c. bouillon	none
1 c.c.	10 c.c. bouillon	1-plus
1 c.c.	11 c.c. bouillon	1-plus
1 c.c.	12 c.c. bouillon	2-plus
1 c.c.	13 c.c. bouillon	2-plus
1 c.c.	14 c.c. bouillon	2-plus
1 c.c.	15 c.c. bouillon	3-plus
1 c.c.	16 c.c. bouillon	4-plus
1 c.c.	17 c.c. bouillon	4-plus
1 c.c.	18 c.c. bouillon	4-plus
1 c.c.	19 c.c. bouillon	4-plus
1 c.c.	20 c.c. bouillon	4-plus

It can be seen from this experiment that the optimum range for blood culture dilutions ranges between 1-15 to 1-20, and conforms to the results obtained in the two experiments previously described. The positive growth was estimated by the turbidity of the medium. Negatives were confirmed by subcultures. After the optimum dilution for blood cultures was determined, it was decided to repeat the experiments using plain agar instead of bouillon in order that the amount of growth might be more accurately checked and to determine differences that may exist between the efficiency of the two types of culture media.

Rabbit No. 4.—Inoculated with 1 c.c. of a saline suspension of Staphylococcus aureus, containing about 10,000 organisms per c.c. Fifteen minutes later 25 c.c. of blood was withdrawn from the heart and planted out on the following quantities of plain agar melted to 45° C., and plates immediately poured.

TABLE IV

AMOUNT OF BLOOD	CULTURE MEDIUM	GROWTH
1 c.c.	1 c.c. plain agar	none
1 c.c.	2 c.c. plain agar	none
1 c.c.	3 c.c. plain agar	none
1 c.c.	4 c.c. plain agar	none
1 c.c.	5 c.c. plain agar	none
1 c.c.	6 c.c. plain agar	8 colonies
1 c.c.	7 c.c. plain agar	40 colonies
1 c.c.	8 c.c. plain agar	100 colonies
1 c.c.	9 c.c. plain agar	500 colonies
1 c.c.	10 c.c. plain agar	innumerable
1 c.c.	11 c.c. plain agar	innumerable
1 c.c.	12 c.c. plain agar	innumerable
1 c.c.	13 c.c. plain agar	innumerable
1 c.c.	14 c.c. plain agar	innumerable
1 c.c.	15 c.c. plain agar	innumerable
1 c.c.	16 c.c. plain agar	innumerable
1 c.c.	17 c.c. plain agar	innumerable
1 c.c.	18 c.c. plain agar	innumerable
1 c.c.	19 c.c. plain agar	innumerable
1 c.c.	20 c.c. plain agar	innumerable

It can be seen from the above experiments that low dilutions of blood in solid media produce analogous results as in bouillon, and the figure of 1 part of blood to 20 parts of medium will also be suitable on solid media though smaller quantities of media may be used.

Rabbit No. 5 was inoculated at the same time and with the same dose as the one previously described, and a similar series of culture media plates were poured, except that the blood drawn from the heart was first defibrinated, using mechanical agitation with glass beads for a fifteen-minute period. No growth was obtained on any plate. This would indicate that the organisms were destroyed by the agitation or were destroyed by complementary action during the fifteen-minute period.

From the animal experiments carried out with Staphylococcus aureus, it would seem that at least a dilution of 1-20 is required before maximum efficiency can be obtained. Certainly inhibition of growth occurs if dilutions lower than that figure are used. Therefore, in order to take a blood culture using 5 c.c. of blood, 100 c.c. of medium would be required, this requirement evidently being necessary to adequately dilute the bactericidal substance to prevent marked inhibition of growth. Later experiments to be described indicate that the dilution is no important factor but that this large proportion of plain bouillon is required to bind the complement in that quantity of blood.

The experiments shown up to this point were carried out two years ago by one of us (R. R. K.), and at that time it became evident that the fixation of complement in the patient's blood would be necessary before bacteria that may be present in the blood stream could grow without inhibition from complementary activity. At that time a method toward this end was devised, having for its purpose the utilization of larger quantities of blood free from complement activity. This was done by drawing 50 c.c. of the patient's blood, introducing this into a citrate saline solution in a sterile 250 c.c. centrifuge bottle, centrifuged at a high rate of speed, and the sediment seeded on the

various plain and special media. Fox and Leaman[4] have reported a similar method, and reported 41 per cent positive as contrasted to their previous positive percentage of 25, including 34 cases in their report. After a short trial we discarded this method as being unsatisfactory due to the chances of contamination, special apparatus required, and unsatisfactory results.

Attention has long been called to the bactericidal activity of the normal constituent of the blood stream known as complement. It was first shown by John Hunter that freshly shed blood resisted putrefaction, although at that time the work of Bordet, Ehrlich and Buchner on this subject had not been done. It was not many years later when Lubarsch[7] demonstrated that one drop of normal rabbit serum was capable of killing 50,000 virulent anthrax bacilli in vitro. Buchner found that the blood serum had the same bactericidal power as whole blood and called this substance alexin. Nuttall also showed that various organisms were destroyed by the normal body fluids and that the bactericidal activity was lost by heating to 56° C.

Since that time, a voluminous amount of work has been done on the nature and properties of complement, but as yet the exact chemical nature of this substance is unknown. Sufficient evidence has accumulated, however, to show that complement is probably the most important single factor in the destruction of bacteria by the blood serum, as evidenced by the work of earlier investigators and recently elaborated upon by Cadham[8] who has shown that the normal complement varies within narrow limits during health, but during the course of an infectious disease, it fluctuates widely, the amount always proportionate to the degree and severity of infection. This has also been shown by Hadjopoulos and Burbank.[9]

Since it is generally accepted that the blood contains varying quantities of complement and that normal complement is decidedly bactericidal, it then looks reasonable to assume that if a specimen of blood is withdrawn and cultured for the purpose of growing organisms that this complement in the patient's blood stream will exert an inhibitory effect on the growth, thus in a way, defeating the very purpose of the procedure.

We believe this factor to be of the highest importance in the failure of obtaining more positive blood cultures. This idea is not entirely new as Owen, Martin, and Pitts[10] have reported the use of a method for blood cultures which utilizes the addition of trypsin which has a destructive action on complement, basing their method on the work of Douglas and Colebrook[11] who originally brought this out. Burbank[1] reports that he uses a 10 per cent peptone solution for the fixation of complement, and Cummins[12] in 1922 found that colloidal silica would efficiently fix complement and at that time suggested that it could possibly be used in blood cultures. As far back as 1911 Cummins[13] also brought out the complement-fixing properties of bile, and suggested that the success of this substance in the cultivation of typhoid bacilli in blood cultures was due to this action. Our own experiments to be described in this paper bear out this assumption.

It has been shown that many substances are capable of fixing complement on a nonspecific basis, as Mackie and Finkelstein[14] have shown that complement may be fixed by such substances as peptone, bacterial emulsions,

cholesterol, amino acids, sodium oleate, and colloidal substances. It is also well known that the alcoholic and aqueous extracts of various normal body tissues are capable of fixing complement in varying degree.

After carefully considering the factors brought out before, we began experimental work in an effort to find a satisfactory substance that could be added to the ordinary culture medium, which would efficiently and immediately bind the complement of the patient's blood and yet not inhibit the growth of bacteria, and which could be prepared in a simple and practical way.

EXPERIMENTAL WORK

Technic.—A determination of the complement-fixing value of a large number of substances was made. This was done by using the sheep cell hemolytic system, assuming that the bactericidal activity of the complement would roughly parallel the hemolytic properties. Gordon and Wormal[15] have shown that these two properties are probably identical. The unit used in all titrations was ½ c.c. of a 5 per cent suspension of washed sheep cells, with the necessary unit of anti-sheep amboceptor. Human complement was used throughout. The serum of several bloods was mixed in an effort to provide a uniform unit of complement. The pooled serum was diluted 1-10 and a titration carried out each time to determine the exact complement unit.

The following outline, using 10 per cent peptone as the substance whose complement-fixing qualities are to be determined, as an example, will illustrate the method used in all substances.

Tube	1	2	3	4	5	6	7	8	9	10	11
Normal Salt Solution	0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5 c.c
10% Witte's Peptone, 1.0 c.c., ½ c.c. transferred from tube 1 to 2, 2 to 3, and so on to tube 10, and ½ c.c. discarded from tube 10. Tube 11 control. Complement unit in all tubes. Incubated for one hour at 37° C.											
Hemolytic unit of sheep cells and amboceptor added to each tube.											
Incubation for one hour at 37° C.											

At the end of this time the tubes were centrifuged and the dilution of the peptone in that tube showing a complete absence of hemolysis was taken as the complement-fixing unit of that substance. For example in tube No. 4 or a 1-8 dilution, the unit of complement fixation is 8.

Table V shows the complement-fixing units of the various substances tried.

The series of titrations and filtrates was carried out to determine the complement-fixing properties of this class of substances, in that the phenomenon of anticomplementary Wassermann sera is very frequently noted because of bacterial contamination.

From the series certain indications may be derived:

1. That plain meat extract bouillon has little complement-fixing value, whether it be prepared from fresh meat or meat extract.

2. Fresh beef heart extract has a relatively high fixing power as compared with fresh veal bouillon.

3. There is little difference in the fixing value of killed and unkilled organisms.

TABLE V

	SUBSTANCE USED	TUBE SHOWING COMPLETE INHIBITION	UNIT OF FIXATION
1.	Beef extract bouillon	No complete fixation	Less than 1
2.	Beef heart bouillon	3	4
3.	1% Peptone	2	2
4.	5% Peptone	3	4
5.	10% Peptone	4	8
6.	20% Peptone	4	8
7.	24 hour growth B. Subtilis in bouillon (killed)	3	4
8.	48 hour growth B. Subtilis in bouillon (killed)	3	4
9.	72 hour growth B. Subtilis in bouillon (killed)	3	4
10.	24 hour growth B. Subtilis in 1% Peptone (killed)	2	2
11.	48 hour growth B. Subtilis in 1% Peptone (killed)	3	4
12.	72 hour growth B. Subtilis in 1% Peptone (killed)	3	4
13.	96 hour growth B. Subtilis in 1% Peptone (killed)	4	8
14.	120 hour growth B. Subtilis in 1% Peptone (killed)	4	8
15.	Berkefeld filtrate of Number 10	2	2
16.	Berkefeld filtrate of Number 11	2	2
17.	Berkefeld filtrate of Number 12	2	2
18.	Berkefeld filtrate of Number 13	2	2
19.	Berkefeld filtrate of Number 14	2	2
20.	24 hour growth B. Subtilis in 5% bouillon (killed)	3	4
21.	48 hour growth B. Subtilis in 5% bouillon (killed)	3	4
22.	72 hour growth B. Subtilis in 5% bouillon (killed)	4	8
23.	96 hour growth B. Subtilis in 5% bouillon (killed)	4	8
24.	120 hour growth B. Subtilis in 5% boullion (killed)	4	8
25.	Berkefeld filtrate of Number 20	2	2
26.	Berkefeld filtrate of Number 21	2	2
27.	Berkefeld filtrate of Number 22	2	2
28.	Berkefeld filtrate of Number 23	2	2
29.	Berkefeld filtrate of Number 24	2	2
30.	24 hour growth B. Subtilis in 10% bouillon (killed)	3	4
31.	48 hour growth B. Subtilis in 10% bouillon (killed)	3	4
32.	72 hour growth B. Subtilis in 10% bouillon (killed)	3	4
33.	96 hour growth B. Subtilis in 10% bouillon (killed)	3	4
34.	120 hour growth B. Subtilis in 10% bouillon (killed)	3	4
35.	Berkefeld filtrate of number 30	2	2
36.	Berkefeld filtrate of Number 31	2	2
37.	Berkefeld filtrate of Number 32	2	2
38.	Berkefeld filtrate of Number 33	2	2
39.	Berkefeld filtrate of Number 34	2	2
40.	24 hour growth B. Subtilis in 1% Peptone (living)	4	8
41.	24 hour growth B. Subtilis in 5% Peptone (living)	4	8
42.	24 hour growth B. Subtilis in 10% Peptone (living)	5	16

4. The complement-fixing factors do not pass through the Berkefeld filter; therefore, it is probable that the bodies of the organisms are responsible for the fixation, and not the soluble products of bacterial growth.

5. Because of the low complement-fixing values, and due to the technical difficulties that may arise in blood cultures if dead bacteria were used as the fixing agent, this class of substances is probably unsatisfactory.

6. Peptone has a slight complement-fixing value, but we do not consider it sufficiently high to be of marked practical value.

In the second series, the titrations were carried out in an identical manner, using extracts of various body tissues prepared in the following manner:

Five hundred grams of tissue were freed of fat and ground in a meat grinder, and to this was added 1000 c.c. of water, allowed to stand overnight in the ice box, and then filtered through several layers of gauze, removing the solid particles. The filtrate was then used for the complement-fixing determinations. In the case of liver and brain tissue, we were unable to obtain a

filtrate free from finely suspended meat particles. The results of the unheated filtrates and those heated to various degrees, mixed in varying proportions, are given below:

TABLE VI

SUBSTANCE USED	TUBE SHOWING COMPLETE INHIBITION	UNIT OF FIXATION
Veal extract (unheated)	2	2
Heart extract (unheated)	2	2
Liver extract (unheated)	4	8
Brain extract (unheated)	6	32
Veal extract (56° for 30 min.)	1	1
Heart extract (56° for 30 min.)	1	1
Liver extract (56° for 30 min.)	2	2
Brain extract (56° for 30 min.)	4	8
Veal extract (75° for 15 min.)	0	0
Heart extract (75° for 30 min.)	0	0
Liver extract (75° for 30 min.)	1	1
Brain extract (75° for 30 min.)	3	4
Veal extract (100° for 5 min.)	0	0
Heart extract (100° for 5 min.)	0	0
Liver extract (100° for 5 min.)	0	0
Brain extract (100° for 5 min.)	2	2
Veal extract (15# for 20 min.)	0	0
Heart extract (15# for 20 min.)	0	0
Liver extract (15# for 20 min.)	0	0
Brain extract (15# for 20 min.)	1	1

Results of the above series of titrations indicate that brain extract has a relatively high fixing value, followed by liver, heart, and veal in the order named. Only these four tissues were selected because of their availability for practical use. As indicated before, the possible explanation for the relatively high fixing values for brain and liver is that some meat particles were held in suspension.

It was also noted during the course of heating, that there existed almost a direct proportion between the degree of heat applied and the rapidity of coagulation, and the size of the resultant coagulated meat particles. It was also noted that the complement-fixing values were in proportion to the size of the coagulated meat particles; the finer the meat particles, the greater the complement-fixing value. It was then decided to devise a method of heating and coagulation that would keep the meat particles in a state of fine division. This was done by *slowly* heating to 100° C., *stirring vigorously* during the process. As a result, coagulation became complete with an almost powder-like fineness to the meat particles. The four tissues were then heated as described above, and complement-fixing values determined with the following results:

	TUBE	UNIT
Veal extract (containing meat particles in fine suspension)	5	16
Heart extract (containing meat particles in fine suspension)	5	16
Liver extract (containing meat particles in fine suspension)	6	32
Brain extract (containing meat particles in fine suspension)	6	32

From the above titrations it was readily determined that the fine meat particles in suspension are almost solely responsible for the high degree of complement fixation.

This is in accordance with the work of Cummins[12] who showed that colloidal silica fixed complement to a marked degree and who referred to the work of Mutermilch and Bankowske showing that alumina possessed the same properties.

Although not included in our tables, we carried out complement-fixation reactions on cream, egg-yolk, and bile. In each case we found a complement-fixing value that was relatively high, especially in the case of bile which gave a fixing unit of 128. In our mind this explains the efficiency of the blood culture medium in current use for growing typhoid bacilli, and this is further borne out by the relatively high fixing value of liver substance. For routine blood cultures, however, liver substance should not be used because of the bile solubility of such organisms as the pneumococcus.

Since it was determined that brain substance in suspension had the greatest complement-fixing value, followed by liver and heart, it was then decided to combine brain and heart substance in various proportions, test these mixtures for fixing value, and from this deduce a formula for a suitable medium.

In carrying out these titrations, it was decided to simulate as much as possible the conditions that exist in the blood culture flask; that is, a substance must be used that is not only capable of fixing the complement but one that will bind the complement before it has time to act upon the bacteria, or in other words, a substance that will bind the complement immediately. With this in mind the method of titration was changed to meet this requirement.

Method of Titration.—A series of tubes was set up, the appropriate dilutions of the substance to be tested, then made in the respective tubes. The hemolytic unit, including amboceptor and cells, was then added. At this time, then, we have in the tubes the two complement binding factors, that is, sheep cells and amboceptor, and the substance in question. The complement is then added to each tube and incubation for thirty minutes carried out. Therefore, in those tubes showing no hemolysis the complement had been bound by the substance tested so rapidly that no hemolysis could take place. Following this plan, the tubes containing the larger amounts of substance showed complete inhibition of hemolysis, the latter increasing as the substance decreased. In the table below complete inhibition is indicated by four plus and lesser degrees of inhibition by the respective plus numbers.

We wish to emphasize at this point that the degree of inhibition is less than if a routine complement titration was being carried out.

From Table VII it is seen that bile has the greatest fixing power. For routine blood cultures, however, it must be ruled out. Brain suspension has a high immediate fixing value, and was tested with an equal quantity of heart suspension and found to give complete inhibition in the second tube. As the second tube contains ¼ c.c. of brain substance, then this quantity is capable of fixing the unit of complement completely and immediately. The unit of complement in this case was ½ c.c. of a 1-10 dilution of human serum. We estimated as follows:

½ c.c. 1-10 human complement is fixed by ¼ c.c. medium.
1 c.c. of undiluted complement would be fixed by 5 c.c. medium.

We have estimated that two c.c. of blood contains approximately one c.c. of serum, therefore:

2 c.c. of blood would be fixed by 5 c.c. of medium.
20 c.c. of blood would be fixed by 50 c.c. of medium.

TABLE VII

SUBSTANCE TESTED	TUBES					
	1	2	3	4	5	6
1. Brain suspension (autoclaved)	++++	++++	+++	++	0	0
2. Filtrate of No. 1	++++	++	+	0	0	0
3. Heart suspension (autoclaved)	++	+	+	0	0	0
4. Filtrate of No. 3	++	+	0	0	0	0
5. Veal suspension (autoclaved)	++	+	0	0	0	0
6. Filtrate of No. 5	++	+	0	0	0	0
7.*Dehydrated brain-heart	+++	++	++	+	0	0
8. Filtrate of No. 7	+	+	0	0	0	0
9. Ox bile	++++	++++	++++	++++	++++	+++
10. 10% Peptone (with 0.5% $NaCO_3$)	+++	++	+	+	0	0
11. Brain 50%, heart 50% (autoclaved)	++++	++++	++	+	0	0

*Brain suspension 25 per cent, heart suspension 75 per cent, plus other media ingredients dehydrated.

On this basis, we believe that 50 c.c. of this medium will immediately fix the complement in 20 c.c. of the average human blood, and would then recommend the use of 10 to 15 c.c. of blood to each flask to allow for a margin of safety to insure a certain complete fixation.

Another series of titrations, using the technic as described, was then set up to determine the elements in the medium mainly responsible for fixation, although this is shown in the last table as the filtrates had little fixing value.

As shown in the following table the substances indicated were centrifuged and the sediments tested for fixing value.

TABLE VIII

SUBSTANCE	*TUBES					
	1	2	3	4	5	6
Brain sediment (autoclaved)	++++	++++	++++	+++	++	+
Dehydrated sediment (brain)	++++	++++	++++	+++	++	+
Brain-heart 50-50	++++	++++	++++	+++	++	+

The above results clearly indicate that the finely divided particles composing the sediment are chiefly responsible for the complement-fixing properties of these substances.

THE EFFECT OF SODIUM CARBONATE ON COMPLEMENT FIXATION

Since sodium carbonate has been suggested as a suitable agent for complement fixation, various titrations were carried out to determine its suitability for use in the medium, to be used in conjunction with a mixture of brain and heart suspension as a base.

Using the technic described before for detection of immediate fixation, the following substances were titrated with the results indicated in terms of four plus for complete inhibition of hemolysis.

TABLE IX

SUBSTANCE TESTED	P_H	TUBES					
		1	2	3	4	5	6
1. 1% dextrose bouillon	7.0	2+	1+	0	0	0	0
2. Same with 0.1% sod. carb.	7.6	2+	1+	0	0	0	0
3. Same with 0.2% sod. carb.	8.0	2+	1+	0	0	0	0
4. Same with 0.5% sod. carb.	8.4	3+	1+	0	0	0	0
5. Same with 0.7% sod. carb.	8.4+	3+	2+	1+	0	0	0
6. Same with 1.0% sod. carb.	8.4+	4+	3+	2+	1+	0	0
7. Same with 1.5% sod. carb.	8.4+	4+	3+	3+	2+	1+	0
8. Brain and heart tissue, equal parts, suspension with 1.5% sodium carb.	8.4+	4+	4+	4+	4+	3+	2+
9. Brain heart suspension with no sod. carb.	7.4	4+	4+	4+	2+	1+	0
10. Same as No. 8 and brought to P_H 7.4 with HCl	7.4	4+	4+	4+	4+	3+	2+
11. 1.5% sol. sod. carb. in normal salt solution	8.4+	4+	4+	3+	2+	1+	0
12. 10% Peptone in Nacl		4+	4+	3+	2+	1+	0

1. From the above series of titrations it may be seen that sodium carbonate in normal salt solution has a high complement-fixing value.

2. This is not increased by the same percentage of sodium carbonate in dextrose bouillon.

3. The fixing value of sodium carbonate increases in proportion to the percentage increase.

4. The fixing value of the brain-heart suspension is increased slightly by adding 1.5 per cent sodium carbonate.

5. The reaction seems to play no part in the complement-fixing value since brain heart tissue, highly alkalinized with sodium carbonate then neutralized with hydrochloric acid, has a fixing value equal to the preparation that is not neutralized.

6. The fixing value of 10 per cent peptone is not equal to that of 1 per cent sodium carbonate.

The conclusions above were drawn after the titrations had been repeated several times with slight variations, and Table IX represents a compilation of several series of titrations, each bearing on the various conclusions. Many titrations were carried out on various dilutions of peptone, but the fixing value of this substance is so much lower than sodium carbonate, that it was ruled out as an important factor.

THE EFFECT OF SODIUM CITRATE ON COMPLEMENT FIXATION

TABLE X

SUBSTANCE TESTED	TUBES							
	1	2	3	4	5	6	7	8
1. 4% sodium citrate in normal saline	4+	4+	4+	4+	3+	2+	1+	0
2. 1.5% sodium citrate in normal saline	4+	4+	3+	2+	1+	0	0	0
3. Brain-heart suspension plus 1% sodium citrate	4+	4+	4+	4+	3+	2+	1+	0
4. Dextrose bouillon plus 1% sodium citrate	4+	4+	3+	2+	1+	0	0	0
5. Brain-heart suspension plus 1% sodium carbonate	4+	4+	4+	3+	2+	1+	0	0

Table X represents a summary of the differences in the fixing value of the substances named, as determined by many repeated titrations. From this the following conclusions may be warranted.

1. Sodium citrate is an efficient agent for the fixation of complement.

2. The complement-fixing values of sodium citrate are almost as much as those of sodium carbonate.

3. After mixing with brain-heart suspension, the fixing value of sodium citrate seems to exceed that of sodium carbonate, or at least, equals it.

4. Its advantages over sodium carbonate for use in cultures media include the fact that the reaction is not disturbed and that it serves as an anticoagulant for blood.

PREPARATION OF THE MEDIA

Having determined the substances best fitted for purposes of immediate fixation of complement, it then became necessary to devise a formula and technic for the preparation of a blood culture medium that would fulfil the requirements laid down by Douglas and Colebrook[11] that is, one that will:

1. Immediately and effectively fix the complement in the patient's blood.

2. That will render inactive or destroy the immune bodies that may be present.

3. That is sufficiently nutritious for the most fastidious bacteria.

4. That is simple, practical, with a technic easily carried out.

We believe that sodium citrate is better adapted for the fixation of complement than any substance or chemical tested, because of its high fixing value, that it does not disturb the reaction, and it serves to prevent coagulation of the blood. The latter is an important factor as it has been shown by Walsh,[16] Harmsworth,[17] Solis-Cohn,[18] and Goldworthy,[19] that blood is far more bactericidal after clotting, due to the probable release of large quantities of complement from the leukocytes.

The media should have a very nutritious base, such as the suspension of brain and heart muscle would provide. The suspended meat particles serve to augment the fixation of complement as shown by our experiments and this is in accord with Gordon and Wormall[15] who showed that complement fixation is dependent upon increased surface areas of particles in solution or suspension.

Dunlop[20] has shown that immune bodies are removed from suspension or rendered inactive by contact with inert substances, as other organisms, coal dust, charcoal, powdered glass, etc. On this basis, we believe that this media, with its finely divided tissue particles, will exert a similar action in blood cultures.

The media should contain a sufficient amount of simple sugar to provide the necessary carbohydrate, and peptone for the split protein material. The addition of sodium citrate in a strength of 1 per cent obviates the necessity for the use of sodium chloride. Based on our results, the following technic for the preparation of the media was adopted after many trial procedures were employed and the media tested.

Fresh hearts of beef and beef brains are obtained at the abbatoir. The hearts are trimmed of fat, the muscle ground finely in a small bore meat grinder, then mixed with water, using 500 grams of ground muscle to 1000 c.c. of water. This is allowed to stand overnight in the ice box. The brain tissue

is macerated in a mortar or passed through a meat grinder. This is mixed with water in the same proportion and allowed to stand overnight in the ice box.

The heart muscle infusion is then pressed through four layers of cloth gauze, and the filtrate heated to the boiling point. This heating need not be done slowly and the mixture should not be constantly stirred. It is then passed through a small mesh wire or copper gauze, the filtrate being the heart muscle extract.

The brain infusion is passed through four layers of cloth gauze, and the filtrate slowly heated to the boiling point with constant stirring. This process coagulates the particles of brain tissue and leaves them in a state of fine suspension. This is not filtered and is the brain suspension. The media has the following formula.

Heart muscle extract	75 parts
Brain suspension	25 parts

To this mixture is added:

Sodium citrate	1.0 per cent
Dextrose	1.0 per cent
Peptone	1.0 per cent
Dibasic sodium phosphate	0.5 per cent

The media is heated until the ingredients are in solution, adjusted to P_H 7.4, then placed in 50 c.c. quantities into bottles or flasks or any other container that may be used for blood cultures. We use a rubber capped, 100 c.c., hexagonal, Pyrex bottle for our cultures. The media is then sterilized at 15 pounds pressure for twenty minutes, and is then ready for use.

As much as 20 c.c. of blood may be introduced into the 50 c.c. of medium as our experiments show that the complement contained in that quantity of blood is immediately fixed. We use from 10 to 15 c.c. of blood in each bottle, thereby allowing a margin of safety, assuring the complete fixation of complement. Our cultures are incubated for three weeks routinely, and we always endeavor to take the culture at the beginning of a temperature rise, believing that the chances of obtaining positives are better at that time than at any other. We usually employ two bottles, one being incubated aerobically and the other anaerobically.

RESULTS OBTAINED

We have cultivated many strains of various fastidious bacteria on this media and believe that it possesses very high nutritious qualities. All streptococci, the four types of pneumococci, meningococci, and gonococci seem to grow luxuriantly. In 320 blood cultures, we have obtained 34 per cent positive cultures as contrasted with 10 per cent positives using the plain veal bouillon, and 18 per cent using the heart extract bouillon. Over one hundred cultures have been taken using dextrose veal bouillon as a control in each instance, resulting in 29 per cent positives with our medium and 9 per cent with the controls. The most striking results have been with chronic infectious arthritis with which we will deal in another paper.

We use the medium in tubes for the isolation of Bargen's diplococcus instead of the dextrose brain bouillon tubes as suggested by Rosenow, and find that it serves equally as well.

CONCLUSIONS

1. Current methods of taking blood cultures seem to be relatively inefficient.

2. This inefficiency is probably due to the failure of the organisms to grow because of inhibition by the patient's complement.

3. Animal experiments are offered to show that a high dilution of blood with media is necessary for good results when using plain culture media.

4. Experimental work is introduced showing the complement-fixing values of various media, bacterial suspensions and filtrates, various animal tissues, and chemicals.

5. A formula and technic for the preparation of a medium designed to fix complement immediately, and destroy immune bodies in the patient's blood, is presented and recommended.

6. Results on a small series of blood cultures are presented.

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920 HENRY STREET.

The medium described in this paper is manufactured by and can be obtained from the Digestive Ferments Co., Detroit, Mich. The dehydrated form has a high complement-fixing value and its nutritive qualities are slightly less than the fresh product.

THE DETERMINATION OF NONPROTEIN NITROGEN IN ONE-TENTH OF A CUBIC CENTIMETER OF BLOOD*†

By Arthur K. Anderson, Ph.D., and Stacey F. Howell, M.S.,
State College, Pa.

THE development of accurate methods for the determination of important blood constituents in small quantities of blood should prove of value to those working with small animals, such as rats, where only small quantities of blood are available. Such methods also should be welcomed in the clinical laboratory, especially in those cases where it is difficult to obtain blood from a vein.

There are several references in the literature to micromethods for the determination of nonprotein nitrogen in 0.1 and 0.2 c.c. of blood. Among these we mention those of Peters,[4] Wilhelmj,[7] Ponder,[5] Kleinmann,[3] and Taylor and Hulton.[6] Wilhelmj, and Taylor and Hulton have compared the nonprotein nitrogen content of venous and capillary blood and have found it to be the same.

This paper reports a modification of the Folin-Wu[2] method for the determination of nonprotein nitrogen which requires only 0.1 c.c. of blood. In this modification the sodium tungstate and sulphuric acid are mixed and diluted to form one precipitating agent. The precipitated protein is separated by centrifuging and the final digestion is made in a test tube graduated at 5 c.c.

PREPARATION OF REAGENTS

1. Precipitating Mixture: To 35 c.c. of water add 5 c.c. of 10 per cent sodium tungstate solution and 5 c.c. of 2/3 N. sulphuric acid.

2. Acid Digestion Mixture: To 405 c.c. of water add 5 c.c. of 5 per cent copper sulphate solution, 30 c.c. of 85 per cent phosphoric acid and 10 c.c. of concentrated sulphuric acid. This solution is Folin and Wu's dilute acid digestion mixture diluted 1 to 5.

3. Standard Ammonium Sulphate Solution: Dissolve 0.4716 gm. of pyridine base free ammonium sulphate in water and dilute to a volume of 1 liter.

4. Nessler's Solution: Prepared according to the directions of Folin and Wu.[2]

PROCEDURE

Sterilize a finger tip with alcohol and after allowing it to dry thoroughly, puncture it with a lancet and collect 3 drops of blood on a small watch glass. By means of a Folin[1] capillary pipette, transfer 0.1 c.c. of blood from the

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watch glass to 1.9 c.c. of freshly prepared precipitating mixture in a centrifuge tube, keeping the tip of the pipette beneath the surface of the liquid. Since this pipette is made to hold 0.1 c.c., the blood is washed out by drawing the liquid up and down in the pipette. Finally, the mixture is thoroughly stirred, using the pipette as a stirring rod. In measuring, care should be taken to remove any blood from the tip of the pipette by wiping it with a filter paper or clean cloth. The mixture is allowed to stand for twenty-five minutes and then centrifuged rapidly for five minutes. We have obtained low values if the mixture has not stood for twenty-five minutes before centrifuging.

After centrifuging, transfer 1 c.c. of the clear supernatant liquid to a dry Pyrex digestion tube (13 × 125 m.m.) graduated at 5 c.c and add 0.5 c.c. of the acid digestion mixture. Digest carefully over a microburner, with a continuous shaking. When the solution turns brown, continue the heating over a low flame until the solution becomes a pale green in color. Cool for one minute, add 3 c.c. of water and shake until a uniform solution is obtained. Next add 1.5 c.c. of Nessler's solution, make up to volume with water and mix thoroughly. Compare this in a colorimeter with a standard prepared by placing 3 c.c. of standard ammonium sulphate solution, 10 c.c. of acid digestion mixture, and 50 c.c. of water in a 100 c.c. volumetric flask and mixing thoroughly. Add to this 30 c.c. of Nessler's solution, make up to volume with water and mix thoroughly. The following formula may be used for calculating results:

$$\frac{\text{Reading of standard} \times 30}{\text{Reading of unknown}} = \text{mg. of nonprotein nitrogen per 100 c.c. of blood.}$$

In the above method the proportions of reagents used are the same as in the Folin and Wu procedure, except the precipitating mixture. Keeping this the same would give a final volume of 1 c.c. in the centrifuge tube. This was tried but it was very difficult to separate 0.5 c.c. of clear liquid after centrifuging. By making the final volume 2 c.c., 1 c.c. of clear liquid may be removed easily. Although twice the volume of precipitating mixture is used, it is not diluted, because this concentration is necessary for the complete precipitation of blood proteins. We have obtained high values in using a dilute precipitating mixture.

The acid digestion mixture of Folin and Wu has been diluted 1-5 to facilitate measuring. It was felt that 0.5 c.c. could be measured more accurately than 0.1 c.c.

It is obvious that the method described requires careful technic and accurately calibrated apparatus. One source of error has been the ammonia in the laboratory fumes. Small traces of ammonium salts on centrifuge and digestion tubes may lead to large errors. One must make sure that all apparatus is clean and ammonia-free before using. For color comparisons, a microcolorimeter has proved very satisfactory because of the small amount of sample available; however, any colorimeter with narrow cups is satisfactory.

Table I shows the results obtained with the new micromethod as compared with the Folin and Wu method; it also shows a comparison of values

TABLE I

A COMPARISON OF RESULTS OBTAINED BY THE FOLIN-WU METHOD AND THE NEW MICROMETHOD FOR THE DETERMINATION OF NONPROTEIN NITROGEN ON VENOUS BLOOD; ALSO A COMPARISON OF THE COMPOSITION OF VENOUS AND CAPILLARY BLOOD AS DETERMINED BY THE NEW MICROMETHOD

BLOOD SAMPLE	VENOUS BLOOD		CAPILLARY BLOOD
	FOLIN-WU	NEW MICROMETHOD	
	MG. NONPROTEIN NITROGEN PER 100 C.C. OF BLOOD		
H. W.	35.2	35.1	34.1
	34.9	34.3	35.2
	34.9	35.1	35.2
	Ave. = 35.0	Ave. = 34.8	Ave. = 34.8
O. H.	32.6	32.4	32.4
	32.6	31.1	32.4
	32.6	32.0	30.8
	Ave. = 32.6	Ave. = 31.8	Ave. = 31.5
L. S.	38.2	37.5	38.0
	38.0	37.5	37.0
	38.0	37.5	37.5
	Ave. = 38.1	Ave. = 37.5	Ave. = 37.5
L. L.	33.0	33.3	33.9
	33.3	32.4	33.2
	33.0	34.3	33.2
	Ave. = 33.1	Ave. = 33.3	Ave. = 33.4
C. V.	37.3	35.1	37.0
	36.8	36.4	36.4
	36.8		36.6
	Ave. = 37.0	Ave. = 35.7	Ave. = 36.7
S. B.	29.7	31.3	32.6
	29.3	29.3	32.3
	29.3	29.3	29.7
	Ave. = 29.4	Ave. = 30.0	Ave. = 31.4
P. A.	34.9	35.5	34.5
	35.7	35.2	35.2
	35.5	34.1	33.3
	Ave. = 35.4	Ave. = 34.9	Ave. = 34.3
J. L.	29.6	30.2	30.0
	29.7	29.6	29.6
	30.0	29.7	29.7
	Ave. = 29.8	Ave. = 29.8	Ave. = 29.8

obtained on venous and capillary blood. The results of the two methods agree closely. It is also evident that capillary blood has the same nonprotein nitrogen content as venous blood.

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MICROSCOPIC SLIDE PRECIPITATION TESTS FOR THE DIAGNOSIS AND EXCLUSION OF SYPHILIS*

By B. S. Kline, M.D., Cleveland, Ohio

DIAGNOSTIC precipitation tests for syphilis of unheated serum, heated serum, and defibrinated finger blood described below are more sensitive than the previously described sensitive precipitation test and less sensitive than the previously described very sensitive precipitation test both of which when ++ to ++++ have been found, with rare exceptions, to be diagnostic of syphilis.[1]

Exclusion precipitation tests described below, when negative, more frequently indicate absence of syphilis than the very sensitive precipitation test previously reported.[1]

The diagnostic and exclusion test emulsions are prepared in just the same way as the sensitive and very sensitive emulsions originally described, and the formulae differ but slightly.

PREPARATION OF ANTIGEN EMULSIONS FOR DIAGNOSTIC AND EXCLUSION TESTS OF UNHEATED SERUM

Formula:

0.85 c.c. Distilled water (P_H about 6).
1.0 c.c. 1 per cent cholesterin (Pfanstiehl c.p.) in absolute ethyl alcohol (99+ per cent).
0.1 c.c. Antigen.
2.45 c.c. 0.85 per cent sodium chloride (c.p. or Reagent, Merck) solution (P_H about 6).

For Diagnostic Test

Place about 1 c.c. of the above emulsion in a narrow test tube (12 mm. inside diameter) in a water-bath at 55° C. for fifteen minutes.

The emulsion as soon as heated is ready for use.

For Exclusion Test

Place 4 c.c. of the above emulsion in a narrow test tube (12 mm. inside diameter) in a water-bath at 55° C. for fifteen minutes, then pour into a 3 × 1 inch tube and centrifuge for fifteen minutes (about 1000 r.p.m.). Decant the fluid and with the tube inverted dry the inside of the tube with a cloth almost to the level of the sediment.

To the sediment add 0.7 c.c. of 0.85 per cent sodium chloride solution (P_H about 6). Transfer to a narrow tube for use.

These emulsions kept at room temperature are satisfactory for use for six hours after preparation.

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PREPARATION OF ANTIGEN EMULSIONS FOR DIAGNOSTIC AND EXCLUSION TESTS OF HEATED SERUM

The emulsion has the same formula as that given above for the unheated serum tests.

For Diagnostic Test

Place about 1 c.c. of the emulsion in a narrow test tube (12 mm. inside diameter) in a water-bath at 35° C. for fifteen minutes. (A beaker of water in the usual laboratory air incubator at 37° C. may be used.)

The emulsion as soon as heated is ready for use.

For Exclusion Test

Place 2 c.c. of the emulsion in a narrow test tube (12 mm. inside diameter) in a water-bath at 55° C. for fifteen minutes. Then pour into a 3 × 1 inch tube and centrifuge for fifteen minutes (about 1000 r.p.m.). Decant the fluid and with the tube inverted dry the inside of the tube with a cloth almost to the level of the sediment. To the sediment add 0.7 c.c. of 0.85 per cent sodium chloride solution.

Transfer to a narrow tube for use.

These emulsions kept at room temperature are satisfactory for use for forty-eight hours after preparation.

For routine use of unheated and heated serum tests, 8.8 c.c. of emulsion is made at one time by using double the amounts in the given formula after which the quantities indicated above are placed in four narrow tubes and heated as outlined.

PREPARATION OF ANTIGEN EMULSIONS FOR DIAGNOSTIC AND EXCLUSION TESTS OF DEFIBRINATED FINGER BLOOD

Formula:

0.85 c.c. Distilled water (P_H about 6).
1.25 c.c. of 1 per cent cholesterin (Pfanstiehl e.p.) in absolute ethyl alcohol (99+ per cent).
0.1 c.c. Antigen.
2.2 c.c. of 0.85 per cent sodium chloride (c.p. or Reagent, Merck) solution (P_H about 6).

Pour the emulsion into a narrow tube (12 mm. inside diameter) and place in a water-bath at 35° C. for fifteen minutes. Then pour into a 3 × 1 inch tube and centrifuge for fifteen minutes (about 1000 r.p.m.) and after decanting the fluid and drying the inner wall of the tube, add 1 c.c. of 0.85 per cent sodium chloride solution (P_H about 6) to the sediment. This is the emulsion for the exclusion test.

The diagnostic test emulsion is prepared from the exclusion test emulsion by dilution with 0.85 per cent sodium chloride solution (P_H about 6) as follows:

0.3 c.c. Exclusion test emulsion.
0.3 c.c. 0.85 per cent sodium chloride solution (P_H about 6).

These emulsions kept at room temperature are satisfactory for use for thirty hours after preparation.

The emulsions for the three diagnostic tests give results more sensitive than the "sensitive emulsions" and less sensitive than the "very sensitive emulsions" previously described.[1]

The emulsions for the three exclusion tests give results more sensitive than the "very sensitive emulsions" previously described.[1]

TABLE I

COMPARISON OF SENSITIVITY OF DIAGNOSTIC AND EXCLUSION SLIDE PRECIPITATION TESTS AND PREVIOUSLY REPORTED SENSITIVE AND VERY SENSITIVE SLIDE PRECIPITATION TESTS

HEATED SERUM	SENSITIVE TEST	DIAGNOSTIC TEST*	VERY SENSITIVE TEST	EXCLUSION TEST*
1	±	+	+, ++	
2	−	±, +	+	
3	+	++	++	
4	±	±, +	+	
5	−	+	+++	
6	±	±	++	
7	−	±	+	
8	±	±	+	
9		±, +	+	
10		−	+	
11		±	+, ++	
12			±	++
13			±	+
14			+	+
15			+, ++	+++, ++++
16			+	+, ++
17			+, ++	++
18			±	++
19			−	±
20			++	+++
21			+, ++	+++
22			+	++, +++
23			+	++, +++
24			+, ++	++++
25			+	++

*Emulsions for these tests prepared slightly differently than those described above, but of equal sensitivity as shown by repeated comparison.

It may be seen from Table I that the diagnostic precipitation test of heated serum is more sensitive than the sensitive precipitation test and less sensitive than the very sensitive precipitation test previously reported and that the exclusion slide precipitation test is more sensitive than the very sensitive slide precipitation test previously reported.

DIAGNOSTIC AND EXCLUSION MICROSCOPIC SLIDE PRECIPITATION TESTS FOR SYPHILIS WITH UNHEATED SERUM

1. Place 3 unheated serum test slides each with 3 large and 3 small chambers on a tray. On a second tray place 3 similar slides and place the two trays in a large holder.

2. Into each of the 18 large rings, pipette 0.03 c.c. of 3½ per cent sodium chloride solution (P_H about 6) near the paraffin wall at one point.

3. After the salt solution is pipetted into the large rings, the unheated sera to be tested are pipetted, first 0.05 c.c. into the small ring, then 0.1 c.c. into the large ring (0.2 c.c. are drawn up into the pipette and after the 0.15 c.c. is delivered, the remaining 0.05 c.c. is blown out, the pipette shaken vigorously five times, and then reinserted into the tube of unheated serum).

4. After the sera are pipetted, a small drop (about 0.01 c.c.) of 3½ per cent sodium chloride solution is allowed to fall from a Wright pipette into the serum in each small ring.

5. The slides in the holder are rotated on a flat surface with moderate vigor for one minute.

6. Into the large chambers, one small drop (about 0.008 c.c.) of exclusion test antigen emulsion is allowed to fall from a Wright pipette. Into each of the small chambers a similar sized drop of the diagnostic test antigen emulsion is allowed to fall from a Wright pipette.

7. The slides in the holder are rotated on a flat surface for four minutes.

8. The results are examined at once through the microscope at a magnification of about 120 times (low power 16 mm. objective, eyepiece 12X) with the light cut down as for the study of urinary sediments and recorded in terms of pluses according to the degree of clumping and the size of the clumps.

Any spilling from a chamber makes the reaction therein unsatisfactory and the serum concerned should be retested.

After the unheated serum tests are finished, the sera (with the 1 c.c. pipettes originally used still in the tubes) are heated in a water-bath at 56° C. for one-half hour.

DIAGNOSTIC AND EXCLUSION MICROSCOPIC SLIDE PRECIPITATION TESTS FOR SYPHILIS WITH HEATED SERUM

1. Place 3 heated serum test slides each with 12 small chambers on a tray in a small holder.

2. Into each of the 36 rings, pipette 0.05 c.c. of the heated serum to be tested (18 sera in duplicate).

3. After all the sera are pipetted, one drop of the diagnostic test antigen emulsion (about 0.008 c.c.) is allowed to fall from a Wright pipette into one-half of the sera. Into each of the other 18 duplicate sera a similar drop of exclusion test antigen emulsion is allowed to fall.

4. The slides in the holder are rotated on a flat surface for four minutes.

5. The results, magnified about 120 times, are read at once through the microscope (16 mm. objective, 12X eyepiece) in the same way as are the unheated serum tests.

DIAGNOSTIC AND EXCLUSION MICROSCOPIC SLIDE PRECIPITATION TESTS FOR SYPHILIS WITH DEFIBRINATED FINGER BLOOD

1. Place a finger blood test slide with two small chambers and two finger blood test slides each with a large chamber on a tray. On a second tray place three similar slides and place the two trays in a large holder.

2. Pipette into each of the 4 large chambers on 4 slides 0.05 c.c. of 3 per cent sodium chloride solution (P_H about 6) close to the paraffin wall.

3. Draw up 0.17 c.c. of defibrinated finger blood and pipette 0.04 c.c. into a small chamber and 0.1 c.c. into a large chamber using the pipette to move the fluid to the limits of the inner chamber. Repeat the procedure with blood from three additional cases.

4. After the bloods are pipetted, a small drop (about 0.01 c.c.) of 3 per cent sodium chloride solution (P_H about 6) is allowed to fall from a Wright pipette into the blood in each small chamber.

5. The slides in the holder are rotated on a flat surface with moderate vigor for one minute.

6. Into the large chambers, one small drop (about 0.008 c.c.) of exclusion test antigen emulsion is allowed to fall from a Wright pipette. Into each of the small chambers a similar sized drop of the diagnostic test antigen emulsion is allowed to fall from a Wright pipette.

7. The slides in the holder are agitated on a flat surface for four minutes.

8. Into the large chambers, 5 c.c. and into the small chambers 2 c.c. of distilled water is allowed to fall from a 25 c.c. pipette (graduated in 0.1 c.c. or 1 c.c.).

9. The fluids are agitated by moving the holder very gently (avoid spilling) for one minute.

10. The results are examined at once or at any time within fifteen minutes through the microscope at a magnification of about 120 times in the same way as are those of the tests described above.

MATERIALS FOR THE MICROSCOPIC SLIDE PRECIPITATION TESTS FOR SYPHILIS

Sera.—These are prepared as for the Wassermann test, care being exercised that they contain no red blood cells or foreign material. After the

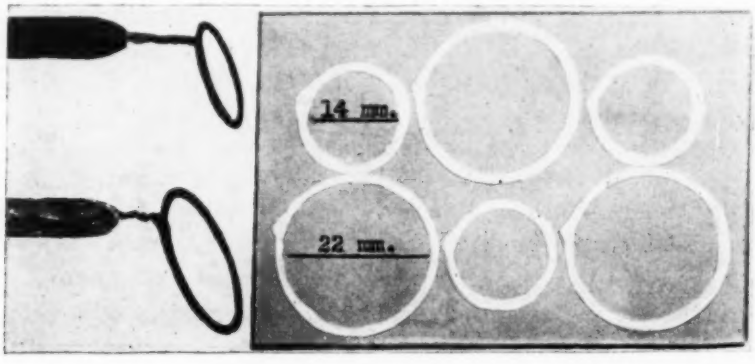

Fig. 1.—Slide for diagnostic and exclusion tests of unheated serum.

unheated serum tests are finished, the sera (with the 1 c.c. pipettes originally used still in the tubes) are heated at 56° C. in a water-bath for one-half hour.

Defibrinated Finger Blood.—The patient's finger tip is cleaned with alcohol, sponged dry, and one or two puncture wounds made with a sharp needle. With the patient's hand in a dependent position, the bleeding finger is squeezed with both hands and the blood allowed to fall into a small vial ($3\frac{1}{2} \times 1\frac{1}{2}$ cm.). The blood in the vial is then stirred with the flat end of a toothpick until defibrinated (several minutes). The vial is stoppered with a cork and properly labelled, and kept in the refrigerator (at about 10° C. preferably) until the blood is to be tested (from a few minutes to twenty-four hours after collection).

Glassware.—Microscopic slides 2 by 3 inches as purchased are rubbed on both sides with Bon Ami paste (prepared by breaking up a cake of Bon Ami in a small quantity of hot water). As soon as the paste is dry (in about five minutes), it is completely removed from the slide with a soft muslin cloth.

For convenience the slides covered with paste may be stuck to each other, allowed to dry and cleaned at any time.

Upon clean slides, for the unheated serum tests, 6 paraffin rings, each with an inside diameter of 14 mm., and 6 with an inside diameter of 22 mm. are mounted. (See Fig. 1.)

For the heated serum tests, 12 paraffin rings, each with an inside diameter of 14 mm. are mounted. (See Fig. 2.)

For the diagnostic defibrinated finger blood test, double ring slides are prepared as follows: Upon the clean slide a steel mold $3\frac{3}{16} \times 2\frac{3}{16} \times \frac{1}{8}$ inches

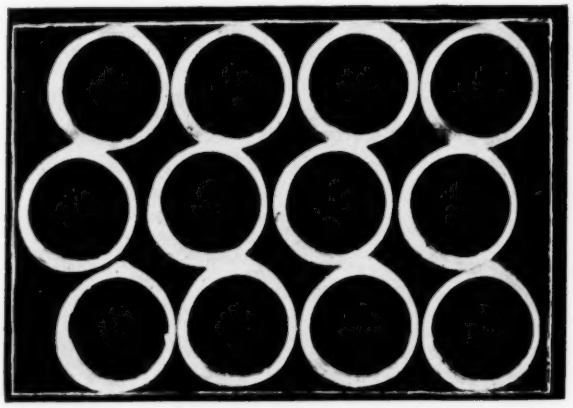

Fig. 2.—Slide for diagnostic and exclusion tests of heated serum.

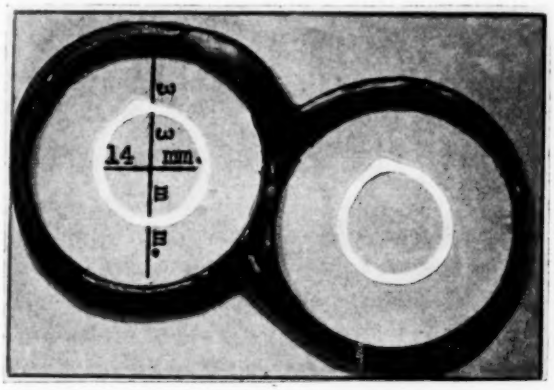

Fig. 3.—Slide for diagnostic defibrinated finger blood test.

with two central wells $1\frac{9}{16}$ inches in diameter is placed. A metal disc $1\frac{5}{16}$ inches in diameter and $\frac{3}{16}$ of an inch thick is then placed in the center of each well. The space between them is filled with hot wax (two parts ordinary vaseline and one part parowax) from a 10 c.c. glass syringe. After the mixture cools a few minutes, the mold is removed by inserting a thin blade between it and the slide. After the outer rings are made (3.3 cm. inside diameter), the inner rings (14 mm. inside diameter) are made with hot parowax by means of a thread wound wire loop, 15 mm. inside diameter. (See Fig. 3.)

The mold for the exclusion defibrinated finger blood test slide is $3\frac{1}{16} \times 2\frac{1}{8} \times \frac{1}{8}$ inches with one rectangular well $2\frac{3}{4} \times 1\frac{7}{8}$ inches. The inner plate is $2\frac{3}{8} \times 1\frac{9}{16} \times \frac{1}{8}$ inches. The space between them is filled with hot wax as described above. The center ring (inside diameter 24 mm.) is made with hot parowax by a thread wound wire loop of proper size. (See Fig. 4.)

Instrument for Making Paraffin Rings.—This is essentially the instrument proposed by Green.[2] A piece of soft iron wire (No. 28) is wound twice tightly about a test tube (about 15 mm. in outside diameter for the loop to make the proper sized rings for heated serum tests, diagnostic unheated serum and finger blood tests; about 23 mm. in outside diameter for the unheated serum exclusion test loop; and about 25 mm. in outside diameter for the exclusion finger blood test loop) forming a double loop and leaving a double shaft about an inch in length. The two shafts are then twisted together to within a quarter of an inch of the free end. After removing the looped wire from the test tube, a piece of linen thread (No. 12) is started

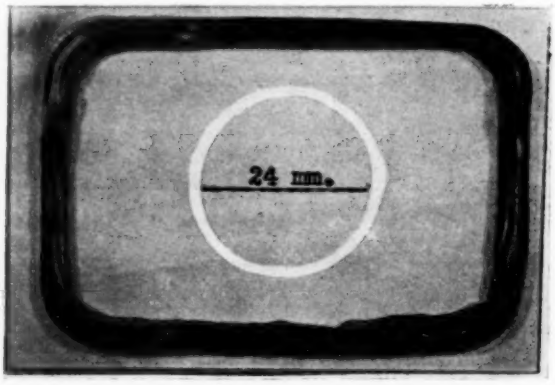

Fig. 4.—Slide for exclusion defibrinated finger blood test.

from the free end of the shaft after being fastened here by a single twist of the two free ends. Three long turns are made reaching the loop which is then tightly wound with the thread. The winding is continued up the shaft to the free end where it is fastened between the two ends of the wire by twisting them. The loop is then bent at right angles to the shaft. It is then reshaped by working the loop against the bottom of the test tube mentioned above. The shaft is then inserted into the handle of a teasing needle or into a straight hemostatic forceps.

The paraffin rings are made by dipping the instrument into smoking paraffin (about 120° C.), draining quickly at one point and transferring the remainder to the glass slide.

Slide Holders.—The holder for 3×2 inch slides is a wooden lid of a slide box ($3\frac{1}{2} \times 6\frac{3}{4}$ inches) containing an easily fitting thin wooden shelf having a small handle at each end. The holder for two slide holders consists of a wooden lid $13\frac{5}{8} \times 3\frac{1}{2}$ inches inside, $14\frac{1}{2} \times 4\frac{1}{2} \times 1$ inches outside measurement.

Pipettes.—The pipettes needed for delivering the sera and defibrinated finger blood and those for preparing the antigen emulsions are the ordinary

finely graduated 0.2 c.c. to 10 c.c. pipettes. The pipette for the 3 per cent and the 3½ per cent salt solution is similar to the 0.2 c.c. antigen pipette. The pipettes for the antigen emulsions are Wright pipettes made from glass tubing 6 to 10 mm. in diameter with the tube about ½ mm. in diameter, delivering a drop equal to about 0.008 c.c. (62 drops per ½ c.c.). A 25 c.c. pipette graduated in 0.1 c.c. or 1 c.c. is used to deliver distilled water to lake the blood in the finger blood tests.

Salt Solution.—Sodium chloride (c.p. or Reagent, Merck) solutions used in the tests are 0.85 per cent, 3 per cent, and 3½ per cent. These are prepared with distilled water having a P_H of about 6. (Such water gives a lilac color when one drop of Chlorphenol Red indicator [LaMotte] is added to 0.25 c.c. of it in a small chamber. Distilled water having a P_H of 5.2 or less gives a yellow color with this indicator and is not satisfactory.)

Antigen.—The antigen is a lipid obtained from chilled absolute ethyl alcohol extract of beef heart muscle powder by precipitation in acetone at 50° C. to 37° C.[3]

Antigen Emulsions.—(See formulas above.) The antigen emulsions are prepared as follows:

Into a one-ounce bottle the required amount of distilled water (P_H about 6) is pipetted. (The P_H of the water need not be determined for the preparation of heated serum test emulsions.)

The bottle is held at an angle and the 1 per cent cholesterin* in absolute ethyl alcohol (99+ per cent) is allowed to run along the side of the neck of the bottle.

The bottle is gently rotated from the neck for twenty seconds.

The bottle is held at an angle again and the proper amount of antigen is pipetted against the side of the neck from a finely graduated pipette.

The bottle is promptly stoppered with a cork and shaken vigorously (the fluid thrown from bottom to cork and back) for one minute.

Lastly, the 0.85 per cent sodium chloride solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken as previously for one minute.

The emulsions, when examined through the microscope, at a magnification of about 120 times, show numerous very fine aggregates but no clumps whatever.

The emulsions are heated at water-baths at temperatures stated above.

For the exclusion tests and for the diagnostic finger blood test, the antigen emulsions are centrifuged in a colloidal gold tube (3 × 1 inch) for fifteen minutes at moderate speed (about 1000 r.p.m.). The supernatant fluid is gently decanted and while the tube is still inverted, the inner wall is sponged with a dry cloth almost to the level of the sediment. The required amount of 0.85 per cent sodium chloride solution is then added, the tube is shaken gently and the emulsion is poured into a narrow test tube for easier handling.

*Prepared in about forty-five minutes by placing in an oven at 50° to 56° C. and shaking gently a few minutes at fifteen minute intervals.

TABLE II

COMPARISON OF THE VARIOUS DIAGNOSTIC AND EXCLUSION TESTS

	DIAGNOSTIC TESTS*					EXCLUSION TESTS*				
	AGREEMENT	RELATIVE AGREEMENT	AGREEMENT AND RELATIVE AGREEMENT	DISAGREEMENT	TOTAL TESTS	AGREEMENT	RELATIVE AGREEMENT	AGREEMENT AND RELATIVE AGREEMENT	DISAGREEMENT	TOTAL TESTS
Unheated serum and heated serum tests	176 Tests 86%	23 Tests 11%	199 Tests 97%	6 Tests[1] 3%	205	178 Tests 87%	22 Tests 11%	200 Tests 98%	5 Tests[2] 2%	205
Unheated serum and finger blood tests	45 Tests 83%	7 Tests 13%	52 Tests 96%	2 Tests[3] 4%	54	27 Tests 69%	12 Tests 31%	39 Tests 100%	0	39
Unheated serum and Wassermann tests	168 Tests 82%	28 Tests 14%	196 Tests 96%	9 Tests[4] 4%	205	166 Tests 81%	28 Tests 14%	194 Tests 95%	11 Tests[5] 5%	205
Heated serum and finger blood tests	49 Tests 87%	6 Tests 11%	55 Tests 98%	1 Test[6] 2%	56	35 Tests 83%	7 Tests 17%	42 Tests 100%	0	42
Heated serum and Wassermann tests	284 Tests 86%	35 Tests 11%	319 Tests 97%	9 Tests[7] 3%	328†	250 Tests 84%	26 Tests 9%	276 Tests 93%	20 Tests[8] 7%	296
Finger blood and Wassermann tests	54 Tests 73%	17 Tests 23%	71 Tests 96%	3 Tests[9] 4%	74‡	46 Tests 77%	11 Tests 18%	57 Tests 95%	3 Tests[10] 5%	60

DISAGREEMENTS:—

[1] 5 negative unheated serum tests, positive heated serum tests.
1 positive unheated serum test, negative heated serum test.

[2] 5 negative unheated serum tests, positive heated serum tests.

[3] 1 negative unheated serum test, positive finger blood test.
1 positive unheated serum test, negative finger blood test.

[4] 7 negative unheated serum tests, positive Wassermann tests.
2 positive unheated serum tests, negative Wassermann tests.

[5] 10 negative Wassermann tests, positive unheated serum tests.
1 positive Wassermann test, negative unheated serum test.

[6] 1 negative finger blood test, positive unheated serum test.

[7] 7 negative Wassermann tests, positive heated serum tests.
2 positive Wassermann tests, negative heated serum tests.

[8] 20 negative Wassermann tests, positive heated serum tests.

[9] 3 negative finger blood tests, positive Wassermann tests.

[10] 1 negative finger blood test, positive Wassermann test.
2 positive finger blood tests, negative Wassermann tests.

*Emulsions for these tests prepared slightly differently than those described above, but of equal sensitively as shown by repeated comparison.
†Approximately 20 per cent of the sera were syphilitic sera giving ± to ++++ results.
‡Over 50 per cent of the finger bloods were syphilitic bloods giving ± to ++++ results.

In addition to increasing the sensitivity of the precipitation tests as outlined above, more sensitive antigens are now employed for diagnostic and exclusion Wassermann tests (Cleveland Method).

FORMULAE OF ANTIGEN EMULSIONS FOR WASSERMANN TESTS OF HEATED SERUM

For Diagnostic Test	*For Exclusion Test*
½ c.c. of emulsion as prepared for finger blood tests (not centrifuged).	0.2 c.c. N. Y. State Health Department cholesterinized antigen.[4]
12 c.c. of 0.85 per cent sodium chloride (c.p. or Reagent, Merck) solution.	10 c.c. of 0.85 per cent sodium chloride (c.p. or Reagent, Merck) solution.
0.1 c.c. to each test.	0.1 c.c. to each test.
(Formerly used for very sensitive Wassermann test.)	

Table II shows a comparison of the diagnostic and exclusion precipitation and Wassermann tests.

Table II shows the excellent agreement of the various tests and the greater sensitivity of the exclusion precipitation tests as compared with the exclusion Wassermann test. In the series there were three cases of chancre of two weeks' duration or less, giving positive results in the precipitation tests and negative results in the Wassermann tests.

The following is a representative protocol showing the sensitivity of the various diagnostic and exclusion tests:*

DISCUSSION

Clinical evaluation of previously reported sensitive and very sensitive microscopic slide precipitation tests for syphilis has shown them to be more valuable when positive as diagnostic of syphilis than indicative when negative of the absence of syphilis. For the purpose of more nearly excluding active syphilitic infection, especially in prospective blood donors, in cases of syphilis under treatment, and in patients presenting themselves with genital and mouth sores, the exclusion precipitation tests described above, when negative, serve better than the very sensitive precipitation test described previously or the exclusion Wassermann test with a very sensitive antigen. In cases of syphilis under treatment, further study will be required to determine the amount and time of treatment necessary after these tests become negative, but the course required will undoubtedly be much shorter than was necessary when less sensitive tests were used as guides.

Because of their great sensitivity, the exclusion tests may occasionally be positive in cases with no evidence of syphilis, and they, therefore, should be used not for the diagnosis of syphilis, but rather when negative, as indicating the absence of syphilis. On the other hand, the diagnostic precipitation tests which are somewhat less sensitive than the very sensitive precipitation test previously employed, when ++ to ++++, may, with very rare exceptions, be relied upon as diagnostic of syphilis.

The precipitation tests requiring the least precautions are those for testing heated sera. In these it is not necessary to determine the P_H of the wa-

*Emulsions for these tests prepared slightly differently than those described above, but of equal sensitivity as shown by repeated comparison.

PROTOCOL 4/2/30

CLINICAL DATA	SLIDE PRECIPITATION TESTS				DEFIBRINATED FINGER BLOOD TESTS		WASSERMANN TESTS	
	UNHEATED SERUM		HEATED SERUM					
	DIAGNOSTIC	EXCLUSION	DIAGNOSTIC	EXCLUSION	DIAGNOSTIC	EXCLUSION	DIAGNOSTIC	EXCLUSION
A.S., Syphilis I treated	−	−	−	−	−	−	−	−
C.C., Syphilis III treated	+, ++	++, +++	±	++	+, ++	++, +++	++	++++
G.P., Syphilis II treated	++++	++++	++++	++++	++++	++++	+++	++++
C.P., Syphilis III treated	−	+	++	+++	−	±, +	++++	++++
J.J., Syphilis III treated	+	++	+, ++	++	±, +	+, ++	+++	++++
W.L., Syphilis I treated	−	−	−	−	−	−	−	−
E.V., Syphilis II treated	+	+, ++	++	+++	++++	++++	+++	++++
P.A., Syphilis II treated	±	++, +++	++, +++	++++	++++	++++	+++	+++
A.B., Syphilis III treated	+++	++++	++++	++++	++++	++++	++++	++++
J.P., Syphilis I treated	−	−	−	++	±	±	−	−
J.F., Syphilis I treated	−	+	++, +++	++++	++, +++	++, +++	+++	+++
C.S., Syphilis III treated	±	++	±	++	±	+, ++	−	−
L.H., Syphilis I treated	−	−	−	−	−	−	−	−
J.C., Syphilis I treated	−	−	−	−	−	−	−	−
P.M. Syphilis I treated	−	−	−	−	−	−	−	−
F.G., Syphilis I treated	−	−	−	−	−	−	−	−
E.L., Nonsyphilitic	−	−	−	−	−	−	−	−
M.G., Syphilis II treated	++	+++	++	++++	+++, ++++	++++	±	−
L.A., Tabes	++++	++++	+++	++++	++++	++++	+++	++
M.A., Syphilis II treated	++++	++++	++++	++++	++++	++++	+	+++

ter and salt solution and, furthermore, the antigen emulsions are satisfactory for use for forty-eight hours.

CONCLUSIONS

1. Diagnostic precipitation tests for syphilis of unheated serum, heated serum, and defibrinated finger blood described above are more sensitive than the previously described sensitive precipitation test and less sensitive than the previously described very sensitive precipitation test both of which when + + to + + + + have been found, with rare exceptions, to be diagnostic of syphilis.

2. Exclusion precipitation tests described above, when negative, more frequently indicate absence of syphilis than the very sensitive precipitation test previously reported.

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A COMPARISON OF THE WASSERMANN AND KAHN TESTS BY MEANS OF A QUANTITATIVE METHOD*

By Seymour C. Schwartz,† B.S., M.D., Washington, D. C.

INTRODUCTION

MANY workers have reported comparisons of various modifications of the Wassermann test with the Kahn test. The comparisons have been made on the basis of the reactions obtained using the routine diagnostic procedures or "standard" tests. The consensus of opinion is that, in general, there is a high degree of agreement between the two tests; also that the substance in, or altered state of syphilitic serum (commonly referred to as reagin) which causes the fixation of complement in the Wassermann test, probably is responsible for precipitation in the Kahn test.

Since 1925, the Kahn test has been performed in parallel with the Wassermann test at the Army Medical School. After the two tests had been compared by laboratory methods and clinical opinion in this laboratory, which functions also as the serologic laboratory of Walter Reed General Hospital, and in nine other of the larger laboratories and hospitals in the United States

*From the laboratories of the Army Medical School, Washington, D. C.
†Major, Medical Corps, United States Army.
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Army, in 1926 and 1927, both tests were officially adopted by the Surgeon General and required to be performed in parallel in all Army laboratories.[1]

A total of over 48,000 parallel tests, Wassermann and Kahn, have been performed at the Army Medical School. In view of the considerable number of such tests already reported by others, it would be superfluous to report these as the findings do not disagree with those already established. It may briefly be mentioned, however, that in this laboratory, the absolute agreement between the two tests in the last two years is about 98 per cent, while the disagreement (one test strongly positive and the other test negative) is a small fraction of 1 per cent. Some of these latter reactions have been found to be due to technical errors in one test or the other by repeating tests, using the original serum where possible. Others may be accounted for as follows: Technical variations in either test as distinguished from technical error; inhibitory substances in the sera; or, to the lower sensitivity of the Wassermann method used as compared to the Kahn test. Where disagreements have occurred and the cases followed for further serologic, clinical, or autopsy evidence, a "false negative" reaction on the part of one test or the other has occurred much more often than a "false positive" reaction.

QUANTITATIVE TESTS ON SYPHILITIC SERA

There are many different methods for performing quantitative tests on syphilitic sera, either by complement fixation or precipitation. The Vernes[2] test is quantitative with results expressed numerically. Kahn[3] has a quantitative test using a series of serum dilutions with results expressed in terms of "units." Kolmer's[4] quantitative method for the Wassermann test employs five decreasing amounts of serum (by serum dilutions) but is not interpreted by a "unit" system. In 1915 and 1916, Vedder[5] at the Army Medical School, quantitatively tested all sera strongly positive in the Wassermann test by a titration method, testing a series of dilutions of the serum. These sera were reported as containing so many "syphilitic units," one unit signifying complete fixation of complement by 0.1 c.c. of serum (the amount used in the routine test), two units signifying complete fixation by 0.05 c.c., one-half the routine amount, and so on. The routine quantitative tests were not continued at the Army Medical School, but with the advent of the Kahn test, quantitative tests were made by the Kahn method. During the past year, quantitative tests have been made by both methods. These tests are not comparable, either in technic or results, and it was suggested by Vedder that an attempt be made to evolve a technic which will give comparable results in both tests. The technic here described was developed with two objects in mind. First, a simple and practical method by which the quantitative Wassermann and Kahn tests can be performed in parallel as the basis for reports to clinicians. Second, a method by which any two serologic tests for syphilis, previously determined as being clinically satisfactory for routine diagnostic use, can be compared and evaluated by their relative ability to measure the varying amounts of reagin encountered in the sera from proved cases of syphilis. It is with this second consideration only that this paper will deal.

ESSENTIAL FEATURES OF COMPARATIVE QUANTITATIVE TESTS

Before describing the technic adopted, a brief consideration will be given to the features considered essential and desirable in any comparison of serologic tests such as is here described.

a. *One series of dilutions for both tests:* This is primarily to reduce the possibility of technical variation and in part to simplify the procedure. The method of preparing the dilutions should be as simple as possible for the same reasons. The dilutions should cover, in the least practical number of tubes, a reasonably wide range. The method here employed conforms to these requirements and although especially adapted for reporting on individual sera, is believed to be sufficiently accurate to form the basis for an equitable comparison.

b. *Technic of tests must be comparable:* This is best accomplished by using the same technic as used in the routine tests. Inasmuch as the diagnostic value of the tests have been determined by a routine technic, this same technic should be applied to the quantitative tests to obtain a true comparison.

c. *Readings of tests must be on the same scale:* To conform to the readings in general use, we have read both tests on the "four-plus" scale. Although it is possible to assign definite values to the minor differences in readings on this scale, Kahn's simple method of interpretation has been adopted and readings of two-plus or over are called positive and one-plus or less called negative.

d. *Units must be determined on the same system:* Kahn's system of units has been adopted for both tests. This assigns 4 units to a positive reaction in the undiluted serum. The measure of a serum being then 4 times the dilution ratio in which a positive reaction is obtained.

e. *Not too complicated and time-consuming a method:* This is partly for ease of accomplishment but mainly to reduce to a minimum the factors tending to produce technical errors or variations.

TECHNIC

Kahn test.—Kahn's routine diagnostic (three tube) test, using standard antigen prepared in this laboratory.

Wassermann test.—Craig's[6] modification with an antihuman hemolytic system, the method standard in the United States Army. The antigen used is an alcoholic extract of ether extracted dehydrated beef heart containing 0.2 per cent cholesterol, prepared as follows:

Place 25 gm. beef heart (Bacto-Beef Heart, Dehydrated, "Difco") in a 250 c.c. Erlenmyer flask. Add 200 c.c. ether (anesthesia). Extract at room temperature four days, shaking 3 or 4 times daily. Filter. Dry beef heart and flask until free from ether odor and replace beef heart in flask. Add 225 c.c. absolute alcohol. Extract at 37° C. for nine days, shaking 3 or 4 times daily. Filter through fat free paper. To the alcoholic extract add 0.2 per cent cholesterol. Keep in ice box. A flocculent precipitate generally forms in a few days which may be filtered out.

Technic of quantitative tests.—

a. *Inactivation of serum:* Fresh serum inactivated at 56° C. for thirty minutes; serum tested one or more days after inactivation is reinactivated at 56° C. for ten minutes.

b. *Serum dilutions:* For each serum tested use 6 tubes. Place 1.2 c.c. of 0.85 per cent saline in the first tube and 1 c.c. in the remaining tubes. Place 0.8 c.c. serum in the first tube, mix and transfer 1 c.c. to the second tube, continue the transfer to each successive tube, leaving the pipette in the last tube. The undiluted serum is not used as this has been tested in the routine test which has included in the Wassermann test a control on the anticomplementary properties of the serum. The dilutions thus prepared are 1/2.5, 1/5,

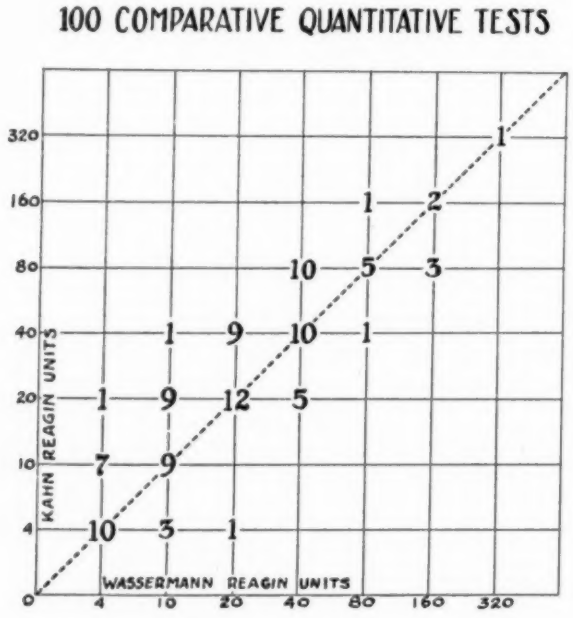

Chart 1.

1/10, 1/20, 1/40, 1/80. These dilutions do not correspond exactly with those recommended by Kahn, but the method has certain advantages. Seldom will a serum be encountered which will give a positive reaction in a dilution higher than 1/80. The addition of the 1/2.5 dilution affects the reagin determination of about 20 per cent of the sera tested and is useful in quantitative tests for determining the effect of treatment as well as furnishing more data for the comparison here attempted.

c. *Kahn quantitative test:* Using the original pipette and starting with the highest dilution, pipette three 0.15 c.c. amounts of each serum dilution into three Kahn tubes containing the three amounts of antigen as used in the routine test. Discard the pipette. Shake and add salt solution as in the routine test. Use the ordinary controls.

d. *Wassermann quantitative test:* With a clean pipette and again starting with the highest dilution, pipette 0.1 c.c. amounts of each serum dilution into a series of 6 Wassermann tubes and perform the test in the same manner as in the routine test, using the ordinary controls, except that the anti-complementary serum controls are omitted.

e. *Reading of results:* Read each test on the "four-plus" scale, the Kahn reading being the average reading of the three tubes. Readings of ++, +++, and ++++ are called positive and +, +− and − are called negative. Assuming in both tests that a positive reaction in the undiluted serum represents 4 reagin units, then a positive reaction in each of the successive serum dilutions represents respectively 10, 20, 40, 80, 160 and 320 reagin units. The potency of any serum is determined according to the formula S = 4 D, where S is the serum potency in terms of reacting units and D is the highest dilution ratio giving a positive reaction.

SPECIMEN PARALLEL QUANTITATIVE TEST

SERUM DILUTIONS		UNDIL.	1-2.5	1-5	1-10	1-20	1-40	1-80
REAGIN UNITS		4	10	20	40	80	160	320
KAHN	Tube 1	++++	++++	++	−	−	−	−
	Tube 2	++++	++++	+++	+	+−	−	−
	Tube 3	++++	++++	++++	+++	+	−	−
	FINAL READING	++++ (Pos.)	++++ (Pos)	+++ (Pos.)	+ (Neg.)	+− (Neg.)	−	−
WASSERMANN		++++ (Pos.)	++++ (Pos)	+++ (Pos.)	++ (Pos.)	+− (Neg.)	−	−

Positive reactions (++++, +++, ++) Negative (+, +−, −)

REAGIN UNITS AS DETERMINED IN ABOVE SERUM,
KAHN = 20 UNITS, WASSERMANN = 40 UNITS.

Chart 2.

100 PARALLEL TESTS

Using the method above described, the results of 100 consecutive parallel quantitative tests are shown graphically in Chart 1. The sera were from cases of syphilis in all stages, both treated and untreated, which gave strongly positive reactions in both the routine Wassermann and Kahn tests. Each test is represented by a single entry on the chart from which may be read the reagin units as determined by either test.

* * * *

In this comparative series, the two tests are in agreement in one-half the tests (49 per cent), while in all other tests except three (3 per cent), the positive reaction in one test is carried out to only one dilution beyond that in the other test. The usually insignificant variations in technic may be greatly magnified in importance in the quantitative tests and this, with the

increased significance given to the readings of partial precipitation or complement-fixation reactions, obviously accounts for some variation in the results, or units determined. For example, reactions similar to that shown in Chart 2 are encountered in which the readings interpreted as positive or negative by a fixed rule do not give a true idea of the close approximation of the results of the two tests. Either test may be apparently favored but in a sufficiently large series these reactions will tend to offset each other.

* * * *

It would be conducive to greater accuracy of comparison if a larger series of dilutions were prepared using dilutions midway between those used in this series, also if a system of determining units were used in which, by evaluating the minor differences in reading the strength of the reactions, results could be expressed in figures intermediate to those here employed. In the method here reported, simplicity is stressed and neither of these refinements have been attempted.

RELATIVE EVALUATION OF THE TWO TESTS

A relative evaluation of the Wassermann test as above described and the Kahn test is made by determining the ratio between the two tests based upon the total number of units measured by each test. In this series, the total number of units measured by the Wassermann test is 3412 and by the Kahn test 3836. If we assume that the Wassermann test is 100 per cent, the Kahn test is then 112.42 per cent. If we assume that the Kahn test is 100 per cent, the Wassermann test then is 88.95 per cent. In other words, in this series, the Wassermann is 11.05 per cent less sensitive than the Kahn, or the Kahn is 12.42 per cent more sensitive than the Wassermann.

As a check the correlation was determined and found to be 0.721 ± 0.122, which is considered significant in view of the small number of tests.

SUMMARY

A simple method is described for determining quantitatively the relative sensitivity of a precipitation test as compared with a complement-fixation test for syphilis. The method is also applicable to comparisons of two precipitation tests or two modifications of the complement-fixation test.

A comparison of the Wassermann test, as standard in the United States Army, with the Kahn test is reported by this method. The Kahn test is found to be approximately 12 per cent more sensitive than the Wassermann test.

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AN IMPROVED ANAEROBIC CULTURE DISH

By Robb Spalding Spray, Ph.D.,* Morgantown, West Va.

THE literature on methods of isolating and cultivating the obligately anaerobic bacteria has been very completely reviewed by Hall,[1] with a bibliography of 247 citations. One cannot fail to be impressed by the variety of devices and principles which have been utilized in the attempt to develop a really satisfactory and practical method, especially for the isolation of these organisms.

It is generally admitted that the most certain mode of effecting isolation is by the use of the single-cell pipette. However, the waste of time and materials, and the difficulties inherent in the satisfactory application of this device to the anaerobes, limit its use practically to the specialist in this field. Apparently appreciating these difficulties, all ingenuity has been expended to evolve a plating method by which isolated colonies may be secured, either by poured-plate or by surface inoculation. Some twenty or more have been cited by Hall.

Since such plates are designed to be self-contained units, practically all rely upon the alkaline pyrogallate method of oxygen elimination. Many of them are either cumbersome, or are so complicated that, while inherently satisfactory, they have never been simplified to a point of feasible commercial production, and hence are not available.

Again, in most of such devices the alkali and pyrogallic acid are mixed, and an attempt is made to seal the dish quickly. As a necessary result, however, a considerable, and unknown, amount of reaction occurs before the seal is accomplished. In order to overcome this disadvantage various ideas are resorted to, such as the use of capsules, cardboard or cotton, to keep the ingredients apart until the seal is effected. All of these introduce chance of contamination, and the fact that they have not been generally accepted implies a need of further development.

Of all such devices described, that of McLeod,[2] involving the use of a special plate inverted over a divided porcelain capsule, suggests a possible further modification. This principle has been carried out in the dish here described, but with the further attempt to reduce it to such simplicity that it may be produced on a commercial basis at a reasonable price. This we feel has been accomplished, and these dishes are now available† for use. (Fig. 1.)

The dish, as illustrated, is composed of two parts; the top being the usual 100 mm. Petri dish lid, the bottom being a special annealed deep glass dish with an up-rolled edge forming a moat for paraffin or plasticine seal. The bottom of this dish is divided by an impressed ridge of sufficient height to contain 10 c.c. or more of fluid on each side.

*Professor of Bacteriology, Medical School, The West Virginia University.
†The Fisher Scientific Company, Pittsburgh, Pa.

In practice, cultures are inoculated into melted casein-digest or dextrose agar by the usual dilution method, after boiling the tubes to drive out oxygen. The dishes are inverted, preferably on a chilled surface, and the culture is poured into the lid of the dish. If the medium is first cooled to 45° C. the agar should set within one minute, without formation of condensation water which might interfere with single colony development. The dish is then reversed, and with separate pipettes the two solutions are placed in opposite compartments of the deep dish. (The total volume of the dish and lid is approximately 300 c.c., and for this volume 4 c.c. of 40 per cent pyrogallic acid and 10 c.c. of 20 per cent NaOH are usually considered amply sufficient.) The moat is then sealed with hard paraffin or plasticine, after which the dish is tilted to mix the solutions, and is incubated at the desired temperature.

Each dish is a self-contained unit which may be examined independently daily until satisfactory colony growth is obtained. The colonies stand out sharply against the dark background of the pyrogallate solution, and with properly reflected light may be examined with the wide-field binocular, and suitable colonies may be marked off for later fishing.

Fig. 1.

By all of such methods, of course, mixed colonies may result from plating mixed cultures. However, at least the commonly studied anaerobes have colony characteristics by which the experienced worker recognizes them, within certain limits, and we have repeatedly deliberately mixed Cl. welchii, Cl. tetani, and Cl. botulinum A, and have recovered pure cultures of each by fishing selected typical colonies.

Objections have been raised to the use of any modification of the pyrogallate method on the ground that CO_2 is essential to all bacterial growth, and that the excess of alkali removes all CO_2, hence inhibiting, if not preventing growth. Admitting that such inhibition is undoubtedly the case, the fact remains that, in the presence of dextrose, the anaerobes with which we have worked grow abundantly, including Cl. tetani which is not known to ferment dextrose. Growth commonly develops as subsurface colonies, of which those near the surface of the agar frequently extend to the surface and produce more or less spreading surface growth. Even though the free CO_2 may be completely absorbed from the gaseous content of the dish it is quite probable that within the medium, in the immediate vicinity of the actively growing organisms, there is sufficient CO_2 to permit satisfactory growth.

Bearing upon this point we have experimentally reduced the amount of NaOH to its minimum requirement. So far experiments have been limited

Fig. 2.—Cl. welchii, two-day culture.

Fig. 3.—Cl. tetani, four-day culture.

to Cl. welchii, which is, of course, the most easily cultivated of the anaerobes. We have reduced the NaOH to 10 c.c. of 2.5 per cent, and the pyrogallic acid to 10 c.c. of 5 per cent, and have observed on several occasions that such

dilutions appear to effect more favorable conditions than do the greater concentrations.

Although little or no information seems available upon the nature of the reactions involved in the formation of alkaline pyrogallate, it has been stated that CO_2, together with acetic and other acids are formed. We have frequently observed that, when the various weaker solutions are mixed, a solution of decidedly varying color results. This may suggest a complex reaction which may produce particularly favorable conditions in certain concentrations, as noted above. It is for such quantitative studies, which we are now pursuing, that we feel this type of dish is eminently suitable, there being, as far as we are aware, no other available device.

The dish offers also opportunity for photographic studies, or for the effect of sugars, or of various chemical reducing substances in the medium such as phosphorus, iron salts, cysteine hydrochloride among others. It would appear to offer further unlimited opportunity for study of comparative merits of media, or of hydrogen-ion concentration, since conditions of anaerobiosis can be quantitatively maintained in all dishes. Studies bearing upon some of these factors are under way at present, the results of which will be presented later.

We have just had occasion to apply the dish to a practical test in a case of postoperative gangrenous infection, in which Cl. welchii and a terminally sporulating anaerobe have been isolated from a mixture of streptococci and paracolon types. We feel that for such purposes, in the small hospital laboratory, without the complex vacuum and hydrogen apparatus essential to intensive research in anaerobic studies, this dish is particularly adapted. The ease and rapidity with which one or more dishes may be set up is a factor of no little importance.

SUMMARY

We have presented a description of an anaerobic culture dish of simple design which has demonstrated its utility for experimental and practical purposes, and has been made commercially available at a reasonable price.

It is a self-contained unit, allowing perfect seal, with complete separation of the reagents until seal is effected.

On suitable media the common pathogenic anaerobes develop isolated colonies from which observations may be made, and isolation of pure cultures effected.

It offers research possibilities in many lines which have heretofore been impractical of development.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LEUCOCYTES: The Differential Count in Infancy, Smith, C. H. Am. J. Dis. Child. 40: 505, 1930.

The conclusions following are based on a comparison of supravital and fixed smear methods.

The differential blood count of thirty-seven normal and sick infants was studied from a supravital preparation and from fixed smears, and the observations were correlated. In forty-three of forty-eight comparative smears, the percentage of polymorphonuclear leucocytes was higher, and that of lymphocytes was lower in the living preparation than in the fixed film.

The average of twenty-four counts from the group of normal infants showed the percentage of polymorphonuclear neutrophils to be 8.6 per cent higher in the supravital than in the fixed coverslip smear and almost 12 per cent higher than in the slide. The lymphocytes, on the other hand, ran 14 per cent and 11 per cent higher in the fixed smear of the slide and coverslip, respectively, than the corresponding cells of the supravital preparation.

The principal reasons for this discrepancy are unequal distribution of the cells and, especially, failure to identify and include all fragmented polymorphonuclear leucocytes in the differential count of the fixed smear. With the supravital technic, the spread of cells is more even, trauma is reduced, and both motile and dying cells may be more readily identified.

The lymphocyte has always been regarded as the predominant cell in the blood of the infant. This observation has heretofore been based on differential counts employing the fixed smear with some modification of the Romanowsky stain. While counts made from the supravital film confirm the preponderance of lymphocytes over polymorphonuclear leucocytes, it is often by no means as marked as indicated in the fixed smear. The differential count from the fixed smear may unduly exaggerate the lymphocytic percentage and convey an erroneous impression of a blood dyscrasia.

In pyogenic infections, especially when associated with a leucocytosis, the differential count from the fixed smears gives at times a much lower polymorphonuclear and higher lymphocyte percentage than expected. That this discrepancy is often due to an increased fragility of the polymorphonuclear leucocytes is evident from comparative supravital studies. In a routine differential count from the fixed smear, particularly when it is employed as an index of the patient's resistance, the extent of white cell damage encountered on the slide should be noted. If fragmented cells have been included in determining the individual cell percentages, mention of this fact should also be made.

MENINGITIS: Diagnostic Value of Sodium Fluorescein in Epidemic, Bonar, B. E., and Bailey, L. G. Am. J. Dis. Child. 40: 3, 1930.

The authors studied the test proposed by Jervell, who stated that the dyestuff was found in the spinal fluid only in meningitis.

The method used was as follows:

The dye was given by mouth or intramuscularly. In both instances, the dosage was the same, 0.025 gm. per kilogram for infants and young children, and 0.030 gm. per kilogram for older persons. When given orally, the sodium fluorescein was mixed with milk or marmalade, and the cerebrospinal fluid was removed at the end of two and one-half hours, by either spinal or cisternal puncture. With intramuscular injections, a 20 per cent solution was used, followed by removal of spinal fluid at the end of one-half hour. The latter method was feasible in most cases. Within a short time after injection, the patients became

progressively yellow and then brown, the peak being reached at the end of four or five hours. Large amounts of the dye appeared in the excreta.

For reading the fluorescence of the spinal fluid, Jervell devised a box that he termed a fluoroscope, unfortunately, since this easily leads to confusion of it with the instrument used in roentgenology. It consisted of a dark box with a light shining into one end through a biconvex lens at a distance of 15 cm. from the tube containing the fluid to be tested. At the other end was an aperture for observation of the tube. The authors' dark box was essentially the same, except that the light was allowed to enter through the top of the tubes, one containing the spinal fluid and one containing water for a control. This simpler form of box served the purpose adequately. Readings were taken first with the undiluted fluid, and then with the fluid diluted until no fluorescence could be detected. It was found that thick, purulent fluids were sometimes difficult to read until centrifuged or allowed to settle; then readings of the supernatant portion were made.

Cerebrospinal fluids containing the dyestuff, sodium fluorescein, were characterized by the following observations: When small amounts were present, the fluid showed no fluorescence in the direct light, but when placed in the dark box a definite light greenish-yellow fluorescence was visible. Fluids containing higher concentrations of sodium fluorescein appeared yellow, and sometimes the fluorescence was apparent in direct light. When these fluids were placed in the dark box, they became a beautiful fluorescent opal color. The fluids were diluted with distilled water until the fluorescence disappeared. The last dilution in which fluorescence occurred was considered the end-point of the test, and this was designated by the number of dilutions. Thus the symbol four-plus (4+) meant that the spinal fluid was fluorescent to and including the fourth dilution. Plus (+) meant that fluorescence could be demonstrated only in the undiluted spinal fluid. A dirty green color without fluorescence was found a few times. Jervell considered this indicative of a minimum amount of sodium fluorescein. The authors found that fluorescence frequently became visible in such fluids after the removal of the thick fibrin and pus. Blood-tinged fluids, if positive, were considered so, since Jervell found that blood in amount up to 0.33 c.c. per ten cubic centimeters did not affect the readings. The authors' observations roughly substantiate this.

They conclude that the test is of value in the differentiation of epidemic cerebrospinal meningitis from other diseases in which sometimes there are manifestations of meningeal irritation, such as pneumonia, erysipelas, spasmophilia, encephalitis, tumor of the brain, and tetanus.

The presence of small amounts of blood does not invalidate the test, which makes it especially valuable in the diagnosis of meningitis.

INDOL: The Detection of in Bacterial Cultures, Duff, D. C. B. Am. J. Pub. Health 20: 1012, 1930.

The author describes a method by dialysis.

Cellophane was the dialyzing membrane used. This material has been described by Hill, and by Wilson, as a sterilizable dialyzing membrane useful in bacteriologic procedures. The organism to be tested is inoculated into 5 c.c. quantities of tryptophane broth in 5″ × ½″ lipless tubes. After forty-eight hours' incubation, the cotton plugs are removed and an unpunctured sheet of cellophane, about 6″ × 6″ is folded down tightly over the mouth of each tube by means of several turns of a rubber band. The cellophane tube or bag thus formed is then slid part way off the test tube, still remaining bound to it by the rubber band. The test tube is now inverted, allowing the culture fluid to fall into the dialyzing bag. The inverted test tube with its dependent bag is then hung within an 8″ by 1″ tube containing 5 c.c. of a saline solution isotonic with the medium. The unit is placed in the ice box for twenty-four hours. The bags are removed, and any fluid adhering to the outer surface is washed down into the dialysate with a few drops from a wash-bottle. The clear dialysate is then pipetted off in 6″ × ⅝″ tube, in which it is subjected to the test. Comparison with color standards is facilitated if the chloroform extract is pipetted off into a still smaller clear glass tube which is placed in a comparator with the standards.

Ice box dialysis is particularly necessary in the case of cultures of motile organisms, which may at higher temperatures penetrate the membrane in such numbers as to cloud the reaction. Formalin as an immobilizing agent is useless, since even in very low concentrations it has the effect of almost completely inhibiting the reaction. Other antiseptics were not tried.

Clough has given a comprehensive review of the various tests for indol and skatol. He recommends a modification of the Ehrlich test. This has given complete satisfaction and for convenience is reproduced below.

Reagents:

(*a*) Paradimethylaminobenzaldehyde, 2 gm. in 100 c.c. of 95 per cent alcohol.

(*b*) HCl, 600 c.c. concentrated plus 200 c.c. water.

(*c*) Chloroform, U. S. P.

Method:

To 5 c.c. of the test solution add 0.5 c.c. reagent (*a*) and 1 c.c. reagent (*b*). Place in boiling water-bath for twenty seconds, shaking vigorously, then place in ice water one-half minute, and extract with 1 c.c. reagent (*c*).

Comparison is made with standards made in the same way.

EXTROSE: Preparation of for Parenteral Injection, Schwentker, F. F. Am. J. Dis. Child. **40:** 533, 1930.

Test tubes 6 × ¾ inches (15.2 by 2.5 cm.) are heated near the open end in a Bunsen flame and drawn out so that they present a constriction near the mouth. Into these, 10 gm. of chemically pure dextrose is poured and allowed to run through the constriction into the tube. The constricted part is then sealed off in an ampule. Ampules so prepared are sterilized by boiling in the water-bath for thirty minutes on each of three consecutive days. When sterile, the ampules may be kept indefinitely, and are made into solution for use simply by dissolving the contents of one ampule in a flask containing the required amount of sterile distilled water. In practice, Schwentker has found it advantageous to keep on hand a supply of flasks containing 200 c.c. of sterile distilled water. The contents of an ampule dissolved in one of these yields 200 c.c. of 5 per cent dextrose solution ready for use; two ampules are dissolved if a 10 per cent solution is required. The dextrose dissolves almost immediately.

Before the ampules of dextrose so prepared were used they were tested in a variety of ways to insure their reliability for clinical use. In order to confirm their sterility, both aerobic and anerobic cultures were made from the contents of a number of ampules. These were uniformly sterile. Finally, a series of tubes were inoculated with spores of the hay bacillus, notably resistant to sterilization, but these were all sterile after being boiled for only the first thirty-minute period in the water-bath.

FLAGELLA: A New Stain, Leifson, E. J. Bacteriol. **20:** 203, 1930.

The stain for flagella described is also an excellent capsule stain. The method of making the smears and staining is exactly that described for flagella staining. The counter stain, however, is obligatory. With basic fuchsin in the stain and methylene blue in the counter stain, the capsules are stained red and the organisms blue.

Organisms such as pneumococci may be stained for capsules by smearing the slides directly from peritoneal fluid. It is, however, generally best to wash the organisms in distilled water before making the smear. The washing is performed as described for flagella staining. The stains are most conveniently made by preparing the smears from distilled water suspensions of the organisms from agar slants.

In case of organisms having both capsules and flagella, both are stained simultaneously. In such cases both the capsules and flagella take the color of the stain while the organisms take the counter stain.

MENINGITIS: Influenzal, Nitrite Reaction as a Diagnostic Test, Greenthal, R. M. Am. J. Dis. Child. 40: 569, 1930.

A few crystals of nitrite-free sodium nitrate were added to a tube of freshly withdrawn spinal fluid. The spinal fluid was incubated for from four to eight hours, and was then ready for the test. First, a few drops of diluted sulphuric acid were added, then, a few drops of diluted potassium iodide solution and then 2 or 3 c.c. of starch paste. If nitrites were present, a deep blue color appeared, due to the formation of free iodine by the nitrous acid. A positive nitrite test could often be obtained early in influenzal meningitis without incubating the spinal fluid with sodium nitrate crystals, but in the later stages of the disease it was necessary to add sodium nitrate so that a sufficient quantity of nitrate was available for reduction to nitrite.

TISSUE: A Microchemical Method for Locating Urease in Tissue, Sen, P. B. Indian J. M. Research 18: 79, 1930.

A small piece of tissue is taken and infiltrated with the following solution for about an hour:

Alcohol	80.0 c.c.
Cobalt nitrate	1.0 gm.
Distilled water	20.0 c.c.

It is then transferred to a solution containing

Alcohol	80.0 c.c.
Cobalt nitrate	0.5 gm.
Urea	0.5 gm.
Distilled water	20.0 c.c.

The tissue is left in the above solution for about forty-eight hours; but if the formation of precipitate is very scanty, it may be left in the solution for about twelve hours more. If the formation of precipitate is very considerable, it should be removed from the solution earlier. The tissue is then prepared for the microtome by the new collodion method or by the paraffin method. The sections are very carefully washed free from soluble salts with distilled water and then treated with a dilute solution of sodium sulphide or a saturated solution of hydrogen sulphide. The deposits of cobalt carbonate in the sections will gradually turn black. After the action is complete they are washed several times with distilled water and mounted permanently in the usual manner. A control section for comparing the changes is prepared by leaving out urea from the cobalt nitrate alcohol solution.

TISSUE: Stain for Tubercle Bacilli in, Moss, E. S. U. S. Vet. Med. Bull. 6: 590, 1930.

Tissues should be fixed overnight in 4 per cent formaldehyde solution, though fair results have been obtained after quick fixation, i.e., by boiling a few minutes in 40 per cent formaldehyde to which a few drops of acetic acid have been added. In spite of this preliminary fixation of the block the treatment of the cut section with 40 per cent formaldehyde is necessary at the beginning of the staining period.

Wash the tissue in tap water to remove the excess of fixing solution. Press the block gently onto the carrier of the freezing microtome with as little water as possible and freeze. There are a few seconds when the block is of the right consistency for cutting, and in that time a number of thin and otherwise satisfactory sections may be produced.

When a number of sections have been cut, they are removed from the knife and dropped into a Petri dish of tap water. For staining, select a thin section, preferably without ragged edges and artificial breaks and tears. Float it onto the slide with the aid of a capillary pipette and bulb and remove the excess of water. It is important to keep the section free from wrinkles and folds during the staining. It is not necessary to attach the sections to

the slide. With a little practice and skill more satisfactory mounts will result with unattached sections.

Proceed with the staining as follows:

1. Flood the section with 40 per cent formaldehyde for eight minutes.
2. Remove and flood with water several times.
3. Slightly overstain with Harris' hematoxylin (about 30 seconds).
4. Flood with water several times, removing the excess.
5. Stain with Ziehl-Neelsen carbol-fuchsin, cold, for eight minutes.
6. Remove stain and flood with 70 per cent alcohol or water.
7. Decolorize to a faint pink with 10 per cent citric acid in 70 per cent alcohol.
8. Flood with water several times.
9. Neutralize in 0.5 per cent lithium carbonate.
10. Flood with water several times.
11. Dehydrate in 95 per cent alcohol.
12. Completely dehydrate and clear in terpinol. (Absolute alcohol may be used.)
13. Completely clear in carbol-xylol. (Phenol 30 parts; xylol 70 parts.)
14. Xylol.
15. Mount in Canada balsam.

DIPHTHERIA: Copper Sulphate Tellurite Medium for Isolation, Allison, V. D. Brit. J. Exper. Path. **11**: 244, 1930.

The medium consists of 2 per cent nutrient agar to which is added 10 per cent of trypsinized horseserum, 0.02 per cent of potassium tellurite and 0.05 per cent of copper sulphate. The trypsinized serum enhances the growth of the diphtheria bacillus, while the potassium tellurite darkens the colonies and gives them a characteristic appearance.

TISSUE: A New Method of Decalcification, Evans, N., and Krajian, A. Arch. Path. **10**: 447, 1930.

The decalcifying solution consists of equal parts of (1) 85 per cent aqueous solution of formic acid and (2) 20 per cent aqueous solution of sodium citrate. This is used following the usual fixation of appropriately sized blocks of tissue in a diluted solution of formaldehyde U. S. P. (1:10) or other standard fixative.

The decalcification requires from a few hours to three or four days, depending on the character of the tissue and the size of the blocks.

After decalcification is complete, the tissue must be washed in running water for from sixteen to twenty-four hours. Thorough washing in water is essential, otherwise the subsequent embedding or freezing preparatory to sectioning cannot be accomplished. The tissue is then cut by the freezing or by the paraffin method and stained in the usual manner.

DEXTROSE TOLERANCE: Modification of, as an Index of Metabolic Activity of the Liver, Althausen, J. L., Gunther, L., Logea, J. B., and Kerr, W. J. Arch. Int. Med. **46**: 482, 1930.

The following modification is used:

In the morning, with the patient fasting, a specimen of blood is taken for sugar determination followed by an injection of 20 units of insulin. Twenty minutes later, 50 gm. of dextrose dissolved in 500 c.c. of water is given to the patient by mouth, followed by 1000 c.c. of water also administered by mouth. After this two samples of blood are taken at intervals of thirty minutes and two more at intervals of one hour. In cases in which insulin reactions developed during the test, the last sample of blood was taken at the time of the reaction regardless of the schedule, and the patient was treated for hypoglycemia in the usual way. Previous to the test, all patients were kept on a general hospital diet for a period of at least several days.

The test has been applied to sixty-four patients with and without clinical evidence of hepatic disease. Only one of these patients experienced difficulty in taking 1.5 liters of fluids, and in no case did the authors meet with an insulin reaction of alarming severity.

The test possesses the following advantages: (*a*) It is simple and requires no special equipment. (*b*) It is harmless to the patient. (*c*) Unlike the levulose and galactose tolerance tests, it is reliable not only in disease groups but also in individual cases. (*d*) It brings out latent functional impairment of the liver and permits one to follow the progress of the disease. (*e*) It checks well with the results of the rose bengal test. (*f*) As a metabolic test, its results are probably independent of biliary obstruction. (*f*) In combination with a dye excretion test, it offers valuable diagnostic aid in toxic cirrhosis of the liver.

The chief limitation of the modified dextrose tolerance test is its inaccuracy in cases of diabetes mellitus with marked morning hyperglycemia.

LEUCOCYTE COUNT: The Normal Filament and Non-Filament Polymorphonuclear Neutrophil Count, Farley, D. L., St. Clair, H., and Reisinger, J. A. Am. J. Med. Sc. **180:** 336, 1930.

The authors divide the cells into filament and nonfilament cells in accordance with the presence or absence of a connecting filament between the nuclear material. They emphasize that the smears must be thin and made on coverslips.

The method, stated briefly, is the differentiation of 100 consecutive leucocytes to determine thus the percentage of non-filament neutrophils, filament neutrophils, lymphocytes, monocytes, eosinophils, and basophils, in which Cooke's criterion is used to separate the polymorphonuclear neutrophils into old and young groups.

The authors wish to emphasize that this method is recommended especially for routine clinical examinations and not in competition with the more complex methods available to expert hematologists.

They have found by this method that the upper limit of a normal young polymorphonuclear leucocyte (nonfilament) count is in the neighborhood of 16 per cent of cells. The average count in 190 presumably normal adults was approximately 8 per cent. An increased percentage of young polymorphonuclear leucocytes may indicate the existence of infection, even though the total number of leucocytes is not increased.

PHOTOGRAPHY: A Method for Direct Contact Printing of Petri Dish Cultures, Buchholz, J. T., and Lewis, I. M. J. Bact. **19:** 105, 1930.

The entire process is carried out in a dark room, using a ceiling lamp as a source of light for printing. At a distance of 2.5 to 3 meters the rays which reach the object, from a concentrated source of light, are very nearly parallel and cast dark, sharp shadows of the colonies on the white paper. The authors have used an ordinary clear glass 150 watt Mazda lamp at 3 meters distance from the object. A special opaque lamp shade is probably of no great advantage, when the ceilings and walls are blackened, but they have used a cylindrical shade painted black on the inside and provided with a 60 mm. opening below in order to prevent reflections from the walls of the room. The light must be centered directly above the exposing table so that the sides of the dish cast no shadows.

All preliminary operations preceding the exposure may be carried out by the use of conveniently placed ruby or amber lights. The Petri dish, with cover removed, is placed bottom side down on the sensitive side of the paper and pressed down. A piece of cloth under the paper facilitates close contact which is necessary. A special plate holder seems to be unnecessary. When plate and paper are properly adjusted the ceiling light is turned on for the desired exposure. The period of exposure depends on several factors and needs to be determined for each culture. The color and transparency of the agar, its depth in the dish, the kind of paper, the developer used, and the temperature of development all influence the time required. With an agar which is almost colorless, and with vigorous Novagas paper,

the exposure may be as short as ten seconds but with No. 4 Azo the same subject may require one hundred and fifty seconds. Agar or gelatin which has more of the amber color, due to beef extract or beef infusion, requires a longer exposure. The time of exposure is so adjusted that development of the print is completed in thirty-five to forty seconds.

The kind of paper used is the most important single detail. Bromide papers are too fast, requiring such short exposures (usually about one-half second) that there is difficulty in properly timing the exposure, and they also lack contrast. If Azo paper is used it should be No. 4 or No. 5; Nos. 1 and 2, in our experiment, always gave unsatisfactory results; No. 3 may be useful for an unusual subject. Excellent results were also obtained by using Gevaert's Novagas vigorous paper. This paper has the advantage of requiring a shorter period of exposure. Extra vigorous Novagas was not tested but is probably still more desirable for very transparent subjects. Both Novagas and Azo papers give satisfactory detail and contrast.

The kind of developer appears to be of considerable importance. The standard Eastman **"MQ" Elon Hydrochinon** developer is less satisfactory than the Eastman Special, which may also be obtained in tubes. The authors have tested several formulae and found the following very satisfactory:

FORMULA D72. STOCK SOLUTION

Water	500.0 c.c.
Elon (Photol or Metol)	2.7 gm.
Sodium sulphite	40.0 gm.
Hydrochinon	10.6 gm.
Sodium carbonate	75.0 gm.
Potassium bromide	0.8 gm.
Water to make	1.0 liter

For use take 1 part stock solution to 1 part water.

FORMULA D73. STOCK SOLUTION

Water	500.0 c.c.
Elon (Photol or Metol)	2.7 gm.
Sodium sulphite	40.0 gm.
Hydrochinon	10.6 gm.
Sodium carbonate	75.0 gm.
Potassium bromide	0.8 gm.
Water to make	1.0 liter

For use take 1 part stock solution to 2 parts water. Formula D72 gives warmer tones and works faster than D73, but either of these formulae will give good results. D73 diluted 1:2 seems to work faster than the stock solution. For a convenient quickly prepared developer, Eastman's Special is recommended.

Sensitized plates or films may be used in the same manner as paper if desired. Since these have a bromide emulsion they must be handled only in ruby light, and their time of exposure is very short, only a fraction of a second, with a less powerful ceiling lamp. A slow emulsion is preferable. The authors have used Eastman's Process films, developed with a contrast developer (D11) with very satisfactory results. The time of development may be varied in order to give different degrees of contrast. These films give positives in the same sense as the contact prints described above, and the prints made from them are reversed, black colonies against a white background. If white colonies against a black background are desired from such films, it is necessary to print the film image in a printing frame or another film which is developed and used as a negative for the final prints. When films are used in making the first impression the differences due to the variations in the thickness of the agar in various parts of the Petri dish are less pronounced. Otherwise the use of films offers no great advantages.

ACETONURIA: Its Value as a Diagnostic Test in Pregnancy: Study of 300 Cases. Bulloch, J. L. J. Okla. State M. A. 23: 121, 1930.

The test is based upon the fact that the nonpregnant woman when deprived of carbohydrates requires at least forty-eight hours to produce acetone, while the pregnant woman requires less time.

The test:

A breakfast consisting of cereal with cream and sugar, buttered toast, and coffee with cream and sugar, or a glass of milk, at 8:00 A.M. After this breakfast, no further intake of food until 5:00 P.M. At that time, another urine specimen is voided, and the first (before breakfast) specimen, and the last (5:00 P.M.) specimen are sent to the laboratory. It is important to impress upon the patient that "no food" means absolutely nothing but water. No chewing gum or "picking" is allowed, from breakfast until the 5:00 P.M. specimen is passed. The specimens are tested for acetone by a modification of the Rothera test which is accurate and easy of manipulation. The technic is as follows: One gram of sodium nitroprussid is weighed out and placed in a mortar. Five hundred grams of c.p. ammonium sulphate is weighed out, and a little of the latter is placed in the mortar with the sodium nitroprussid. The two are finely ground and intimately mixed. A little more of the ammonium sulphate is added and mixed. This is repeated, adding about a teaspoonful at a time, until about 100 grams of the ammonium sulphate is thoroughly impregnated with the sodium nitroprussid. The mixture is poured out upon a large sheet of paper, the rest of the ammonium sulphate is added, and the whole intimately mixed. This mixture is kept tightly corked in a wide-mouthed bottle. The test proper is as follows:

About 1 inch of the mixed nitroprussid and ammonium sulphate is poured into a clean, dry test tube. About three inches of the urine to be tested is added, the whole inverted several times to mix and dissolve. Two c.c. of full strength ammonia water is floated upon the surface of the mixture and allowed to stand for a few minutes. Acetone is demonstrated by a violet to purple color developing at the junction of the two fluids. The deeper the color, the stronger the acetone present.

A positive test for pregnancy is shown when the first tube is negative for acetone and the second tube is positive. (The first tube, being negative, shows that there was no acetone normally in the urine.)

A negative test for pregnancy is shown when both tubes show no acetone. When both tubes show the presence of acetone, there is an acidosis that must be eliminated before the test can be applied.

Of 300 cases studied, 257 were positive, and 43 negative. Of the 43 negative, 40 were negative clinically, and upon examination six months after test. Two were ectopic pregnancies, which ruptured and were operated upon. One was examined three months after test, and was clinically negative, another was lost sight of. Of the 257 positives, 7 premature deliveries, all living; 21 induced abortions, afterward hospitalized; 16 definite histories of miscarriage, doctors being in attendance; 1 uterus full of clotted blood, careful examination showing no evidence of fetus; 212 normal deliveries.

The positive test which was afterward curetted, and showed no trace of a fetus, was the only apparent fallacy in the series, excepting the two ectopics, which gave negative tests.

HODGKIN'S DISEASE: The Blood Picture, Falconer, E. H. Calif. & West. Med. 32: 83, 1930.

From a study of 20 cases the author states that later in the disease, beyond the first year, the leucocyte count tends to become increased, with an increase in the polymorphonuclear leucocytes. Also there is an average and fairly constant increase in the mononuclear cells. The eosinophil count averages about normal or below but may occasionally be very high, reaching in one instance 80 per cent of the total leucocytes.

CALCIUM: Micromethod for, Mazza, F. P., and Rassi, A. Bull. Soc. Ital. biol. sper. **4**: 1217, 1929.

Place 1 c.c. of the neutral solution containing the calcium (0.1 to 4 mg.) in a centrifuge tube, add 1 c.c. of a 20 per cent solution of ammonium sulphate or sodium sulphate free of carbonates, and 1 c.c. of 95 per cent alcohol. Heat on the water-bath for three to four minutes, and centrifuge the solution while hot for two minutes at 3000 r.p.m. Siphon off the clear liquid, and wash the precipitate twice with 2 c.c. of hot 50 per cent alcohol, centrifuging each time and decanting the supernatant. Dissolve the precipitate in cold water and transfer this through a funnel under the surface of a measured volume of 0.01 N iodine acidified with dilute hydrochloric (for amounts of calcium less than 1 mg. 10 c.c. of 0.01 N iodine acidified with 2 to 3 c.c. dilute hydrochloric will suffice). Shake and titrate with 0.01 N sodium sulphate using starch as an indicator. The method has been used for determining calcium in blood serum.

SPINAL FLUID: Quantitative Estimation of Protein, Young, G. A., Bennett, A. E., Christlieb, J. M., and Myers, J. I. Arch. Neurol. & Psychiat. **23**: 542, 1930.

The method is a modification of that described by Bennett and Young in 1926.

To 1 c.c. of spinal fluid in a Hopkins' vaccine tube add 2 c.c. of Tsuchiya's reagent (phosphotungstic acid 1.5 gm.; HCl conc. 5 c.c.; ethyl alcohol q.s. to 100 c.c.).

After standing ten minutes, centrifuge five minutes at 2000 r.p.m. Each 0.01 c.c. graduation on the tube represents 10.3 mg. of protein.

TUBERCULOSIS: Blood Culture in, Loewenstein, E., and Russeff. Wien. klin. Wchnschr. **43**: 294, 1930.

After many futile attempts, the following technic proved satisfactory: 10 c.c. of blood are collected in 5 c.c. of a 5 per cent sodium citrate solution under absolutely sterile conditions and then centrifuged. The plasma is decanted. The blood corpuscles are dissolved in water with 3 per cent acetic acid and are again centrifuged. The stromata are then placed directly and after brief preliminary treatment with 15 per cent sulphuric acid upon this new nutrient medium with the pipette, and then distributed with a platinum loop. (The author succeeded in preparing a pepton-free egg media which proved to be far better than the usual nutrient media. The peptone was replaced by asparagin as a source of nitrogen.)

After four to eight weeks, the tubercle bacilli then became visible even macroscopically. Microscopic examination of the test tubes in which no growth appeared is also recommended, for occasionally tubercle are nevertheless found.

MALARIA: Iron-Hematoxylin Method for, Sinton, J. A., and Mulligan, H. W. Indian J. M. Research **17**: 1329, 1930.

1. Make thin smears of blood on large cover glasses (3″ × 1″).

2. Fix with osmic acid vapor. (This is not essential.)

3. Immerse the blood film while still moist in Carnoy's fluid (absolute alcohol 6 parts, chloroform 3 parts, glacial acetic acid 1 part; this mixture should be freshly prepared) for five to ten minutes.

4. Wash in one change of absolute alcohol and leave in absolute alcohol for a few minutes.

5. If it is desired to remove the malaria pigment, transfer through 95 per cent alcohol to alkaline alcohol (80 per cent alcohol 96 parts, 1 per cent caustic potash solution 4 parts) for several hours.

6. Pass down through graded alcohols to water.

7. Mordant in Bordeaux Red solution (1 per cent aqueous solution of Bordeaux Red) for twelve to twenty-four hours. (This is not essential.)

8. Wash well in water.

9. Mordant in iron-alum solution (4 per cent solution of the violet crystals of iron-alum in distilled water) for from three to eighteen hours, preferably the latter.

10. Rinse quickly in water and place in an aqueous solution of Heidenhain's iron-hematoxylin (1 per cent Heidenhain's hematoxylin in water prepared by the method described by Shortt (1922) for from three to eighteen hours.

11. Rinse in water and differentiate in ½ to ¼ per cent iron-alum solution.

12. Counterstain with eosin, pass through graded alcohols to xylol, and mount in balsam or euparal.

ALTERNATIVE METHOD OF FIXATION

As an alternative to Carnoy's fluid, Gilson's mercuro-nitric mixture (corrosive sublimate) 5 gm.; nitric acid (approx. 80 per cent) 4 c.c.; glacial acetic acid 1 c.c.; alcohol (70 per cent) 25 c.c.; distilled water 220 c.c.; prepare the solution and filter it after three days (this solution keeps well); may be used for fixation and dehemoglobinization of blood smears. Films fixed in this solution and subsequently treated with alcohol become completely dehemoglobinized and the delicately fixed parasites stain well with iron-hematoxylin. The results obtained by this method were quite as good as, and in some cases better than, those obtained after fixation with Carnoy's fluid.

TECHNIC

1. Make thin blood smears on large cover glasses (3" × 1") and while still moist fix in Gilson's fixing fluid for fifteen to thirty minutes.

2. Transfer to 50 per cent alcohol for a few minutes. This completes dehemoglobinization.

3. If it is desired to remove the malaria pigment before staining, the films are treated with alkaline alcohol as before.

4. The remainder of the technic is the same as that described in the previous method.

RAPID METHOD

It will be seen that the technic described above is a somewhat lengthy procedure and in order to curtail the time taken an attempt was made to employ the method of staining by hematin described by Dobell (1914). This method can be used after fixation by Carnoy's and Gilson's solutions but the results obtained were, on the whole, less satisfactory than those obtained after the longer method described above.

TECHNIC

Proceed as in the first method until step 6 is reached.

6. Wash well in 70 per cent alcohol.

7. From 70 per cent alcohol transfer to 1 per cent iron-alum in 70 per cent alcohol (dissolve 1 gm. of the violet crystals of iron-alum in 23 c.c. distilled water [warmed] and add 77 c.c. of 90 per cent alcohol) for at least ten minutes.

8. Rinse in 70 per cent alcohol and place in 1 per cent hematin in 70 per cent alcohol for ten minutes.

9. Rinse in 70 per cent alcohol and differentiate in the iron-alum solution.

10. Pass up through graded alcohols to xylol and mount in balsam or euparal.

TRANSFUSION: Fatal Post-Transfusion Reactions, Brines, O. A. J. A. M. A. **94:** 1114, 1930.

In a series of 4,000 transfusions of unmodified blood, the mortality was 0.05 per cent. Incompatibility of blood was apparently not a factor in the production of these reactions.

Cross agglutination of the blood of the donor and that of the recipient is superfluous provided the direct matching is properly done.

The universal use of group IV donors is strongly advocated as a means of preventing accidents and reducing the incidence of post-transfusion reactions.

The blood group of an individual remains constant throughout life.

The negligible mortality directly attributable to blood transfusion compared with the results obtained argues well for the efficacy and safety of this form of treatment.

ACHYLIA: Neutral Red Test, Streicher, M. H. J. A. M. A. **95**: 326, 1930.

Streicher, from a study of the neutral red test proposed by Glaessner and Wittgenstein in 1923, concludes that the neutral red test may be used as an aid in differentiating true and false achylia gastrica, not because the dye is employed in this test but because a prolonged period is necessary to perform the test.

Neutral red tests may be advantageously employed as a test of gastric function, just as any fractional method may be employed over a long period of time (more than sixty minutes).

Neutral red possesses little or no property as a stimulant of gastric acidity.

YAWS: Epidemiology and Pathology of, Wilson, P. A., and Mathis, M. S. J. A. M. A. **94**: 1289, 1930.

From a study of 1423 cases the authors conclude that:

1. Yaws is, in a large percentage of cases, a disease of childhood; 61.9 per cent of the cases reported occurred in children under ten years of age, and 19.2 per cent in the second decade.

2. Breaks in the skin are the usual port of entry of infection, as evidenced by the predominance of the lower extremities as the site of the mother yaw. Practically, peasants go barefoot most of the time.

3. There is a certain predilection of the moist, exposed surfaces to primary infection. The genitals of Haitian country children are as much exposed as the lips.

4. Yaws becomes less prevalent the higher the altitude above 2,500 feet. This may be due to the more abundant insect carriers at the lower level.

5. The mother yaw leaves a scar which usually persists throughout the life of the individual.

6. It is believed that yaws is occasionally the cause of aortitis and aneurysm.

7. It is believed that yaws is occasionally the cause of cerebral hemorrhage in young adults.

8. Though not proved, the authors believe that yaws is occasionally hereditary.

9. Mucous buccal and pharyngeal patches yielding a spirochete have been found in 3.7 per cent of cases of secondary yaws eruption.

10. All patients with "saber shins" gave a history of yaws in early childhood.

11. There appears to be a regional limitation of tertiary yaws lesions in approximately 11.1 per cent of cases. All patients having the mother yaw on one leg had the tertiary lesion on the same leg.

12. The secondary period fails to occur in about 1.4 per cent of cases.

13. The time interval between the appearance of the primary lesion and the secondary eruption is extremely variable, usually about two to four weeks, but not infrequently this is much prolonged.

14. The secondary eruption is occasionally of the maculopapular type.

15. Yaws may occasionally cause ptosis.

16. Yaws may be an occasional cause of blindness, optic neuritis, and perhaps atrophy.

17. Arsenic-resistant cases of yaws occur occasionally.

18. Acetarsone seems to be the arsenal of choice for general rural clinic treatment of yaws.

BILIRUBIN: Estimation of in the Differential Diagnosis of Cerebrovascular Accidents, Cheney, G. Am. J. M. Sc. **180:** 24, 1930.

On the basis of Wilder's work and the cases herein reported by the author he believes that it seems justifiable to conclude that a definite increase in the icteric index with a negative direct Van den Bergh reaction following a cerebrovascular accident usually indicates that a hemorrhage has occurred, and that a normal index after the first day, but within the first few weeks, is strong evidence against a hemorrhage. If these conclusions are borne out by other workers in larger series of cases where autopsies are obtained, estimations of serum bilirubin will present a most important basis for the differential diagnosis of the lesions.

VAN DEN BERGH REACTION: A New Interpretation, Snyder, H. F., and Reinhold, J. G. Am. J. M. Sc. **8-L2:** 248, 1930.

The authors conclude that the type of direct Van den Bergh reaction observed in serum depends upon the concentration of bilirubin.

In the cases reported an immediate Van den Bergh reaction was associated with a high icterus index and a delayed Van den Bergh with a low icterus index.

The Van den Bergh reaction changed from negative to delayed, then to biphasic, and finally to immediate as increasing amounts of bilirubin were added to human serum.

Dilution of a jaundiced serum with normal serum changed the van den Bergh reaction of the former from immediate to delayed.

The temperature of the reacting materials was shown to be an important factor.

BLOOD COUNTS: Cleaning of Glassware, etc., Van Walsen, G. C. Nedecl. Jijdschr. v. Geneesk. **28:** 3258, 1929.

The author uses the following cleansing fluid: 10 per cent sodium hydroxide 2 c.c., to which is added 2 drops of bromine. The pipettes are filled and allowed to stand ten minutes and then washed with water, alcohol, and ether.

P. BOVISEPTICA: A Simple Mineral Agar for, Scott, J. P. J. Bact. **20:** 9, 1930.

The method of preparing this medium is as follows: Grind 1 pound of beef heart free from fat, or lean meat, through a meat grinder, add 500 to 1000 c.c. water, and set in ice box for fifteen hours. Steam for one hour, cool until the fat hardens and decant. This broth should be used at once or sterilized in 2000 c.c. flasks for future use. When ready to be used, this meat decoction should be filtered through cotton till clear and to it should be added the following:

Peptone	20	gm.
Agar (Difco Bacto purified)	15 to 20	gm.
Potassium citrate	2	gm.
Potassium bicarbonate	0.5	gm.
Ammonium dihydrogen phosphate	0.6	gm.
Ferric ammonium citrate	2.5	gm.
Glucose not more than	0.5	gm.
Distilled water to make	1000	c.c.

Steam for one hour to dissolve the agar, adjust to P_H 7.2. Usually not more than 20 c.c. of normal sodium hydroxide are required. Do not filter. Tube and sterilize at 10 pounds pressure for forty-five minutes.

Difco proteose peptone slightly increases the longevity of Pasteurella organisms grown on this medium.

This mineral mixture agar made without potassium citrate was found to contain a slight precipitate after sterilization, but was slightly superior to the complete mixture for the cultivation of old dried up cultures of Pasteurella boviseptica. The dried growth should be first washed off with sterile saline solution before transfers are made.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Determinative Bacteriology**

THE first edition of this work was published in August, 1923, and represented an initial effort on the part of the committee on Determinative Bacteriology of the Society of American Bacteriologists to place in the hands of students a detailed key for the identification of species based upon a classification of bacteria into families, tribes, and genera. A second edition of the work appeared in December, 1925, which embodied corrections of the previous work and amended descriptions of individual species. The work has become the standard authority in its field and of great practical value to all laboratories, students, and teachers engaged in bacteriologic work.

The third edition of Determinative Bacteriology was published in January, 1930. This new volume represents a notable tome, greatly amplified with descriptions for some two hundred additional organisms not included in previous editions. These new organisms fall chiefly in genera Phytomonas, Flavobacterium, Pseudomonas, Lactobacillus, and Bacillus. Two additional tribes and four additional genera are also recognized. Of great interest is the division of Genus Eberthella placing the dysentery and the typhoid organisms in separate groups. Furthermore the new Manual has attempted to arrange the genera of tribe Bactericae in a more logical manner and a new key for this tribe has been constructed.

In seven years, through three different editions, the *Manual of Determinative Bacteriology* has gained a place of preeminence as an authoritative work on bacteriologic classification and Professor Bergey and members of the committee are to be congratulated for this very important and most practical contribution to the science of bacteriology. No laboratory or scientific library is complete without it.

*Bergey's Manual of Determinative Bacteriology. By David H. Bergey, Professor of Bacteriology, University of Pennsylvania. With an Index by Robert S. Breed. Third Edition. Cloth, 589 pages. The Williams and Wilkins Co., Baltimore.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

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EDITORIALS

The Present Status of B. C. G. Vaccine

IT IS a matter of common knowledge that a large number of those coming to autopsy show healed lesions of tuberculosis evidencing first, the implantation of living tubercle bacilli early in life, and, second, the development of adequate protection.

The dream of the clinician and laboratory worker for many years has been the deliberate production of active immunity to tuberculosis in childhood by deliberate vaccination. So much work has been done with so little result in the past, and so many glowing hopes and claims have failed of substantiation that it is not remarkable that the reopening of the subject by Calmette and his collaborators is received with perceptible apathy and even skepticism.

Nevertheless, the subject is one of such importance as to deserve consideration and the purpose of this review is to present briefly the present status of this procedure.

Clinical, experimental, and autopsy observation have amply established that adult resistance to tuberculosis arises from "tuberculization" of human

beings during childhood; that it occurs in early childhood, and that, despite ignorance of the infecting dose, when it is administered, or of the results produced or the mechanism whereby protective immunity occurs, it is effective to the degree that only approximately 10 per cent of those infected succumb to the disease.

This clinical conception of enhanced resistance to superinfection in tuberculosis through previous infection has been amply confirmed by extensive investigations in cattle by numerous workers.

Its application to the human being, for obvious reasons, has been approached with extreme caution.

Among the first to propose the vaccination of children with living tubercle bacilli was Hamburger[1] who suggested in 1909 the use of small doses (0.000001 to 0.00000001 mg.) of attenuated cultures in children of tuberculous families.

In 1911 Webb and Williams[2] using the Barber technic by which a single bacillus could be manipulated, inoculated two children with weekly doses starting with one and ending with one hundred and fifty bacilli. In 1913 another child was similarly inoculated by Webb and Gilbert.[3]

The results were indefinite, one of the most significant being the development of tuberculous adenitis in the last subject.

It is hardly within the scope of this review to more than mention the difference of opinion relative to the antigen most satisfactory for the production of immunity in tuberculosis. Theoretically, or perhaps in fact, the most effective is the use of living bacilli balanced, if not outweighed, however, by the dangers attendant upon their use.

On the one hand, as pointed out by Petroff[4] although, by the use of living cultures, immunity may be produced by the use of extremely small amounts of material, on the other hand, the inoculations must be continued. There must persist, therefore, a focus of living organisms, an actual infection. Until it is shown conclusively that avirulent cultures *remain* avirulent in the human body, and that their virulence will remain unchanged in the presence of intercurrent infections, the price paid for active immunity thus produced is excessive if not prohibitory. Petroff believes, therefore, that the use of dead tubercle bacilli or their derivatives is at least safe if it has not yet been shown to be effective.

The problem at hand is not the establishment of *absolute* immunity to tuberculosis. This is impossible. What is aimed at is the development of *relative* immunity to the usual small infecting dose the entrance of which is the usual cause of the disease in the human being.

The most extensive effort in this direction so far is that inaugurated by Calmette and his collaborators by means of the so-called B. C. G. (Bacillus Calmette-Guerin) vaccine.

The organism in question is of bovine type first isolated in 1908 which because of environment and long culture on a potato-bile medium (according to Calmette) has entirely lost its power to produce generalized tuberculosis.

This loss of virulence—*when the original conditions of culture are maintained*—has been substantiated by various workers.

Very recently, however, in accordance with modern studies of bacterio-

logic mutation, Petroff and Branch[5] have reported the occurrence in a culture received from Calmette of "R" and "S" type colonies varying in their growth characteristics as well as in their biologic properties. While both types produce tuberculin, both induce hypersensitiveness to tuberculin in guinea pigs, and both produce precipitin and complement-fixing antibodies, the "R" colony always produces tubercles healing in time, while the "S" colony invariably produces progressive fatal disease in the guinea pig.

A somewhat similar investigation in animals casting further doubt upon the absolute harmlessness of B. C. G. is that of King and Park.[6]

Confirmation and amplification of these observations is obviously of the greatest practical importance.

Following a prolonged study of the immunizing effect of inoculation of this attenuated culture in cattle, Calmette suggested its application to the immunization of children.

The vaccine is prepared either by cultivation on the original glycerin-potato-bile medium or on a synthetic fluid medium (Sauton's). The growth, not over three weeks old, is then made into a homogenous suspension in a solution containing 1 per cent dextrose and 4 per cent glycerin in water and standardized so that one cubic centimeter contains five milligrams moist weight of bacilli.

The vaccine is administered orally, two centimeters of suspension in milk, and fed on the third, fifth and seventh days after birth. Each feeding represents 400,000,000 living organisms.

The principles upon which the procedure is founded—and which Calmette believes to have been established by his researches—are thus summarized by Long[7]:

Immunity to tuberculosis can be produced only by living tubercle bacilli which must retain their essential characteristics.

To be of any value at all vaccination must be practiced before infection, which, for all practical purposes, means within the first ten days of life.

The vaccination of infants is best carried out by oral administration as the intestinal mucosa in the first few days of extrauterine life is readily permeable to bacteria.

The living tubercle bacillus used for vaccination must be so far attenuated as to have lost permanently the power to produce anatomical tubercles in susceptible animals and such an organism is available in B. C. G.

A point of essential importance is the *permanent* loss of virulence. Reference has already been made to the observations of Petroff and Branch in this connection and space does not permit discussion of the work of other observers such as Gerlach,[8] Nobel,[9] Kraus,[10] Selter,[11] and others.

B. C. G. vaccine as a prophylactic measure in the human being was begun by Turpin and Weille-Hallé in 1921 in the maternity hospital in Paris, since which time a voluminous literature has accumulated.

Before discussing in a general way the significance of the statistical data which has accumulated, it is of interest to consider the criteria by which the efficiency of protection may be measured.

In the last analysis, of course, the incidence of tuberculosis in the vac-

cinated as compared to the nonvaccinated groups should be the final criterion. The accurate computation of such statistics is a matter of great difficulty, however, influenced by a variety of factors hard if not impossible to control, and related to other factors whose variability cannot be measured.

Among these latter are the varied and unknown size of the infecting dose and the degree and duration of the immunity produced.

It is of interest to discuss for a moment the exact relation of the hypersensitiveness of tuberculosis to immunity. In other words, is tuberculin hypersensitiveness synonymous with or even proportionate to immunity?

This phase of the question has been the subject of wide discussion and an exact parallelism between the two has been both assumed and denied.

All that can be said at present is that immunity is an exceedingly complicated phenomenon dependent upon many factors, allergy among them. But, as emphasized by Long, allergy (or hypersensitiveness) and immunity cannot be discussed as if they were unit characters nor can immunity be evaluated accurately solely in terms of the gross allergic reaction which, after all, "is nothing more than a focus of active hyperemia and local edema."

While the relation of tuberculin sensitivity to protection against tuberculosis is a very clouded question to date, that some relation exists is highly probable and it is upon the ground of lack of uniform production of tuberculin sensitivity by B. C. G. that the efficiency of the procedure has been attacked by many workers, especially Selter.[12]

Still further objection is based upon the contention that immunity is a phenomenon contingent upon the biological alteration of tissue, in other words, that it arises from the response or the reaction to cellular drainage. Bacteria, therefore, which produce neither tuberculin sensitivity or tissue reaction in the form of anatomic tubercles cannot be expected, in accordance with this view, to produce immunity.

To date approximately 100,000 children have been treated with B.C.G. vaccine.

While it is established that infants are born with no appreciable resistance to tuberculosis, and that infection takes place most commonly in the first year of life, the prevalence of tuberculosis as established by the tuberculin-skin test varies very considerably throughout the world. It is, apparently, much greater in Europe than in the United States. In Vienna it has been reported as 94 per cent, in Paris 35 per cent and in New York 10 to 12 per cent.

It is apparent that only the most careful, critical, and extensive comparison of vaccinated and nonvaccinated groups will suffice to establish the true value of protective vaccination. So far, such a comparison is not available.

While Calmette's statistical data are en masse imposing, when they are subjected to critical analysis some of their value disappears.

Petroff and Branch[13] comment that not a single group of vaccinations has so far been controlled.

Calmette claims that in France the mortality rate from tuberculosis in the first year of life has been reduced from 25 per cent before vaccination to 0.9 per cent after vaccination.

Petroff and Branch, who, after all, summarize the opinions of many oth-

ers, believe that some, at least, of this reduction may be attributable to the betterment of social conditions coupled with a better understanding of tuberculosis and its manner of dissemination. It is not clear, also, whether this 25 per cent refers to a group of known infected as detected by the tuberculin reaction or to the general mortality. Still further, they comment, the vaccinated children of tuberculous mothers were removed from contact for four months, thus decreasing the likelihood of infection, while this protective measure was not applied to unvaccinated children.

How are these two factors of vaccinated and protective hygiene to be evaluated or compared?

Finally, without autopsy, how is the cause of death in either the vaccinated or unvaccinated groups to be determined?

As said by Long,[14] "It is only fair to state that the experiments themselves, the application of the vaccine, and the collection of the statistics have all been well in the control of a relatively small group. That they are anxious to succeed goes without saying. It is equally significant that every one else wants them to succeed, and in this very fact errors in the interpretation of the results by those less scientifically trained, but licensed to administer the vaccine may well invade the statistics."

There can be no doubt that the method is still in the strictly experimental stage and that, in the last analysis, time will be the deciding factor.

When the procedure has been carefully and extensively tried by a number of workers in animals; when such a trial has shown beyond cavil its uniform and constant harmlessness; when it has still further been tried under the most exacting and controlled circumstances in children, again under the critical observation of many workers; and finally, when these experiments have been carried on for a number (five or ten) of years, then and only then can definite and authoritative opinions be pronounced.

The tuberculin fiasco is not so far distant as to fail to remind us that caution and conservation are still excellent watchwords.

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—*R. A. K.*